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ARTICLE

Effects of Growth Type, Sowing Date, and Sowing Rate on the Canopy Architecture, Protein Yields, and Oil Yields of Winter Oilseed Rape (*Brassica napus* L.)

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ABSTRACT

A split-split-plot design was used to evaluate the effects of sowing dates and sowing rates on three winter rape cultivars, including 'PR45D03', a semi-dwarf hybrid, 'PR46W31', a traditional hybrid, and 'Californium', an open-pollinated cultivar. August 25 was the optimal sowing date for maximizing protein and oil yields across all three cultivars. Of the cultivars, the traditional hybrid, 'PR46W31', produced the highest protein and oil yields on that date. The yields of the semi-dwarf hybrid, 'PR45D03', were greater than those of the open-pollinated cultivar, 'Californium', when these were sown later than the optimal date. Protein and oil yields did not differ significantly among different seeding densities.

1. Introduction

Sowing date and sowing density combined account for 80% of the variation in the yielding potential in winter rape (*Brassica napus* L.) [6, 19]. Advances in winter rape breeding and genetic engineering techniques have made cultivar development the least expensive element of improving yields [1, 18]. According to Bartkowiak-Broda [4], the cultivar was the most important factor in determining the volume and quality of seed yield produced by oilseed rape. Intensive breeding programs have increased the number of winter oilseed rape cultivars, including hybrids, that are being registered in Poland. The biology and yielding of hybrid cultivars with a semi-dwarf habit have yet to be thoroughly investigated. Many studies have been conducted to investigate the effect of sowing

date on agronomic traits, but few of these have been on semi-dwarf hybrids. According to Al-Ahmad [1], the effect of sowing date needs to be tested in these cultivars, as late sowing may be better tolerated by tall cultivars. Cultivation recommendations for sowing date and sowing rate for new oilseed rape cultivars need to be verified. The aim of this study was to determine the effect of sowing date and sowing rate on the dynamics of winter rape growth and development, as well as on yield-forming aspects of the plant habit in various cultivars of winter rape, including an open-pollinated cultivar, a traditional hybrid, and semi-dwarf hybrid.

2. Material and Methods

Field trials with winter oilseed rape (*Brassica napus* L.)

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were conducted during two successive growing seasons, 2008-2009 and 2009-2010, at the Złotniki Experimental Station (N 52° 29.193'; E 16° 20.569') in Poland. The trials were conducted using a split-split-plot design with four field replications and three experimental treatment factors, including sowing date, winter rape cultivar, and sowing rate. Sowing dates were August 14, August 25, September 4, September 15; winter rape cultivars were 'PR45D03' (semi-dwarf hybrid), 'PR46W31' (hybrid F1), and 'Californium' (open-pollinated); sowing rates were 30 seeds·m⁻², 40 seeds·m⁻², 50 seeds·m⁻², and 60 seeds·m⁻².

The experimental plots were 14 m² and were located on sandy luvisol soils deposited over light clay ^[12]. A four-row, single-seed Monosem drill was used to sow seeds in 4 rows with an inter-row spacing of 0.35 m. Nitrogen fertilizer was applied in August, prior to sowing, at a rate of 25 kg·ha⁻¹. Nitrogen fertilizer was again applied at a total rate of 112 kg·ha⁻¹ in two doses in March and April of 2009. Phosphorus and potassium fertilizers were applied at the rate of 21.8 kg·ha⁻¹ and 103.75 kg·ha⁻¹, respectively, and determination of these rates was based on analyses of available forms of both nutrients in the soil's A-horizon. The plots were sprayed with the herbicide Butisan Star 416 SC at a rate of 3 l·ha⁻¹ after oilseed rape sowing; the herbicide Fusilade Forte 150 EC was applied at a rate of 1 l·ha⁻¹ after plant emergence; the herbicide Galera 334 SL was applied at a rate of 0.35 l·ha⁻¹ in the spring of 2009. During each growing season plants were sprayed with insecticides three times: Fastac 100 EC was applied at a rate of 0.12 l·ha⁻¹ in April 2009, Karate Zeon 50 CS was applied at a rate of 0.12 l·ha⁻¹ in April 2009 and in May 2010, and Nurelle D 550 EC was applied at a rate of 0.6 l·ha⁻¹ in April 2009 and 2010. Fungicides were applied twice during each growing season; Horizon 250 EW was applied at a rate of 0.7 l·ha⁻¹ after plant emergence, and Pictor 400 SC was applied at a rate of 0.5 l·ha⁻¹ at the onset of flowering. To ensure uniformity of plant maturation and to prevent shattering in early July, Spodnam DC and Roundup Energy 450 SL desiccant were applied at a rates of 1 l·ha⁻¹ and 3 l·ha⁻¹, respectively.

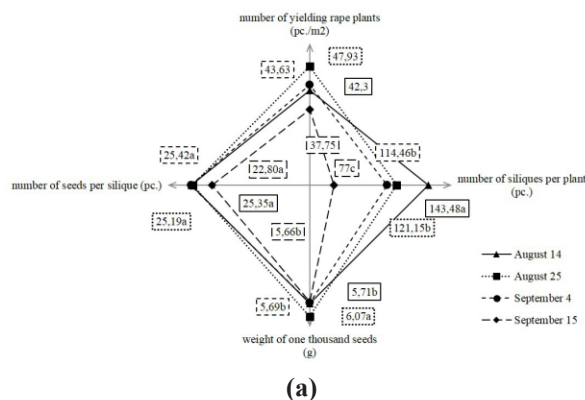
At the stage of seed ripening (BBCH 80-82), a total of 50 siliques per plant, including 25 each from the main and lateral shoots, were collected. The mean number of set seeds in siliques and the number of siliques harvested from an area unit were calculated. After harvest the 1000 seed weight and the weight of 1 hectolitre of seeds were determined using an electronic meter of seed density per storage volume (DRAMINSKI GMDM Electronics in Agriculture, Poland). Protein and oil were determined in the Plant Breeding Laboratory in Strzelce, department Małyszyn, using near-infrared spectroscopy in a NIRSys-

tems 6500 spectrometer (FOSS NIRSystems Inc., Silver Spring, MD).

Yield parameters and biometric measurements were analyzed using analysis of variance (ANOVA) in the Statistica (ver. 10) software package. A principal components analysis (PCA) was used to identify regularities the multi-dimensional analysis.

3. Results

The highest average numbers of plants per unit area were obtained when seeds were sown on August 25. Sowing 10 days earlier decreased the number of yielding plants by 5.6 plants per 1 m², while a sowing 10 and 20 days later decreased the number of yielding plants by 4.3 and 10.2 plants per 1 m² (Figure 1a). The semi-dwarf hybrid 'PR45D03' produced the greatest number of plants per unit area (Figure 1b). The highest number of yielding plants were obtained at the greatest sowing density, 60 seeds·m⁻² (Figure 1c). Plants sown at the earlier date (14.08.) set the greatest number of siliques per plant. Later sowing dates reduced this number by 22.3, 29.0 and 66.5 for the dates of August 25, September 4 and September 15, respectively (Figure 3a). The hybrid cultivar with the traditional growth habit formed 170.3 more seeds per plant than the semi-dwarf morphotype. The number of siliques formed per plant decreased with increasing sowing density. The greatest number of siliques was set by plants sown at a density of 30 plants·m⁻² (Figure 1c). Plants sown at the latest date, September 15, formed fewer seeds per silique, although these differences were not confirmed statistically (Figure 1a). Among the tested cultivars, the semi-dwarf hybrid 'PR45D03' formed the highest number of seeds per silique. Differences in the numbers of seeds per silique were small relative to the cultivars 'PR46W31' and 'Californium', amounting to 0,67 and 0,48 seeds (Figure 1b). The greatest thousand seed weight was produced by the open-pollinated cultivar 'Californium'. Increased seeding density reduced 1000 seed weight by an average of 5,81 (30 pc·m⁻²) – 5,75 (50 pc·m⁻²).



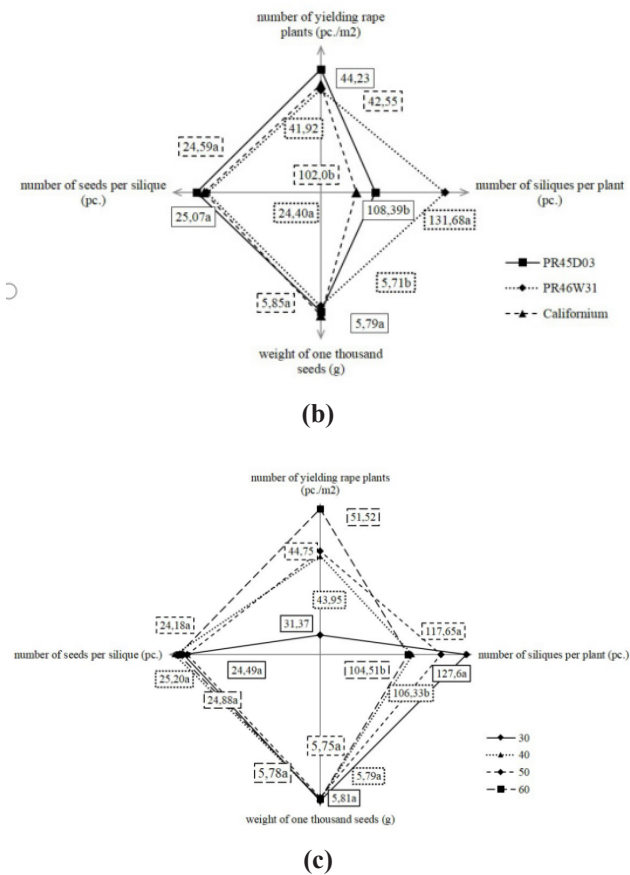


Figure 1. Yield components in winter oilseed rape depending on: (a) sowing date, (b) cultivar, and (c) sowing density.

Note: Means within a columns followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$);

The results of an ANOVA for oil and protein yields are presented in Table 1. The highest yields of total protein (1166.1 kg·ha⁻¹) and oil (2203.2 kg·ha⁻¹) were obtained from plants sown at August 25. Sowing after August 25 significantly decreased the yields of protein and oil in each cultivar. Sowing on September 4 reduced protein and oil yields by 255.3 kg·ha⁻¹ and 518.1 kg·ha⁻¹, respectively. Sowing on September 15 further reduced protein and oil yields by 616.9 kg·ha⁻¹ and 1,267.1 kg·ha⁻¹, respectively, compared to plants sown on August 25. The traditional hybrid, 'PR46W31', produced significantly higher yields of protein and oil than other varieties. The semi-dwarf hybrid protein and oil yields were not significantly different than those produced by the open-pollinated variety, 'Californium'. Protein and oil yields changed were significantly dependent on sowing date and cultivar. The highest protein and oil yields were obtained for all cultivars when sown on August 25. A large decrease in the protein and oil yields occurred in all cultivars when plants

were sown later than the optimal sowing date. Delaying sowing from August 25 to September 15 caused the largest reductions in protein and oil yields in the semi dwarf hybrid, 'PR45D03', and plants of this cultivar produced 525.7 kg·ha⁻¹ (49.2%) less protein and 1155.3 kg·ha⁻¹ (55.1%) less oil when sown on September 15 compared those sown on August 25. Protein yield was significantly increased by increasing the seeding density from 30 to 40 pc·m⁻², and an increase to a sowing density to 60 pc·m⁻² caused a non-significant increase in protein yield (Table 1). The oil yield produced at a seeding density of 30 pc·m⁻² (1,627.6 kg·ha⁻¹) was significantly lower than the yield produced at higher seeding densities. Higher seeding densities produced oil yields ranging from 1712.7 (40 pc·m⁻²) to 1737.3 kg·ha⁻¹ (50 pc·m⁻²).

Table 1. Protein and oil yields (kg·ha⁻¹) depending on sowing date, cultivar and sowing rate

Treatment		Protein yield			
		'PR45D03'	'PR46W31'	'Californium'	Average
Sowing date	August 14 14.08.	920,77 ^{cd}	1115,71 ^{abc}	1013,56 ^{bcd}	1016.7 B
	August 25 25.08.	1067,98 ^{abcd}	1280,60 ^a	1149,67 ^{ab}	1166.1 A
	September 4 4.09.	900,68 ^{cd}	971,91 ^{bcd}	859,70 ^e	910.8 C
	September 15 15.09.	542,25 ^f	588,70 ^f	516,52 ^f	549.2 D
	Average	857.9 B	989.2 A	884.9 B	
		Oil yield			
Sowing date		'PR45D03'	'PR46W31'	'Californium'	Average
	August 14 14.08.	1834,23 ^{bcd}	2193,32 ^{ab}	1917,88 ^{bcd}	1981.8 B
	August 25 25.08.	2096,04 ^{abcd}	2355,26 ^a	2158,32 ^{abc}	2203.2 A
	September 4 4.09.	1700,70 ^{de}	1772,88 ^{cde}	1581,82 ^e	1685.1 C
	September 15 15.09.	940,76 ^f	1021,19 ^f	846,27 ^f	936.1 D
	Average	1642.9 B	1835.7 A	1626.1 B	
Sowing rate (pc·m ⁻²)		Protein yield		Oil yield	
	30 pc·m ⁻²	869.0 ^b		1627.6 ^b	
	40 pc·m ⁻²	916.8 ^a		1712.7 ^a	
	50 pc·m ⁻²	928.2 ^a		1737.3 ^a	
	60 pc·m ⁻²	928.7 ^a		1728.6 ^a	

Note: Means within a columns followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$)

The coefficients of variation calculated for the protein and oil yields of all tested varieties showed that both of the earlier sowing dates (August 14 and 25) guaranteed stable yields compared to later sowing dates (September 04 and 15) (Table 2). The increase in yield variability (CV%) on delayed sowing dates was mainly due to the significant decrease of minimum yields.

Table 2. Statistical characteristics of protein and oil yields

Sowing date	Variety	CV (%)	SD	Values	
				Min	Max
Protein yield					
14.08.	PR45D03	8.3	76.1	816.3	1006.8
	PR46W31	3.3	37.3	1043.3	1157.5
	Californium	10.8	109.4	877.4	1145.5
25.08.	PR45D03	8.7	93.1	926.7	1166.4
	PR46W31	3.1	39.5	1230.9	1346.8
	Californium	6.8	78.8	1004.2	1235.6
04.09.	PR45D03	30.3	272.6	491.1	1202.3
	PR46W31	27.5	267.4	600.6	1244.7
	Californium	26.5	228.0	576.1	1104.0
15.09.	PR45D03	70.6	365.5	126.7	928.5
	PR46W31	78.7	497.9	99.7	1157.5
	Californium	82.0	425.8	83.4	966.6
Oil yield					
14.08.	PR45D03	8.4	155.0	1645.1	2014.2
	PR46W31	3.3	71.9	2075.7	2283.5
	Californium	8.8	168.7	1666.9	2081.7
25.08.	PR45D03	10.9	230.0	1776.6	2325.6
	PR46W31	6.7	157.1	2197.8	2556.8
	Californium	3.8	82.7	2024.5	2247.2
04.09.	PR45D03	30.0	511.0	946.1	2269.9
	PR46W31	26.0	461.1	1137.4	2222.0
	Californium	28.7	454.1	1048.1	2074.3
15.09.	PR45D03	72.0	677.7	215.5	1809.2
	PR46W31	76.2	778.7	181.2	1871.6
	Californium	81.0	685.5	140.9	1614.5

The relationships between seed yield volume and its components was determined for individual cultivars in terms of the selected sowing dates (Figure 2). Regardless of the selected sowing date, a strong relationship was observed between yields and the number of siliques per 1 m^2 in both the semi-dwarf and traditional hybrid. However, yield was correlated more strongly with the number of seeds per silique in plants sown on the last sowing date, September 15. Moreover, a significant negative correlation was found between yield and 1000 seed weight in traditional hybrid sown on August 14 and 25. It is only an apparent contradiction, however, since the basic yield components affect not only seed yield, but also one another. This is frequently manifested in the fact that an increase in the value of one of the components, e.g. silique number, is correlated with a reduction of another, such as

the number of seeds per silique or the 1000 seed weight. Similarly, an increase in the number of seeds per fruit is frequently correlated with reduced weight per seed.

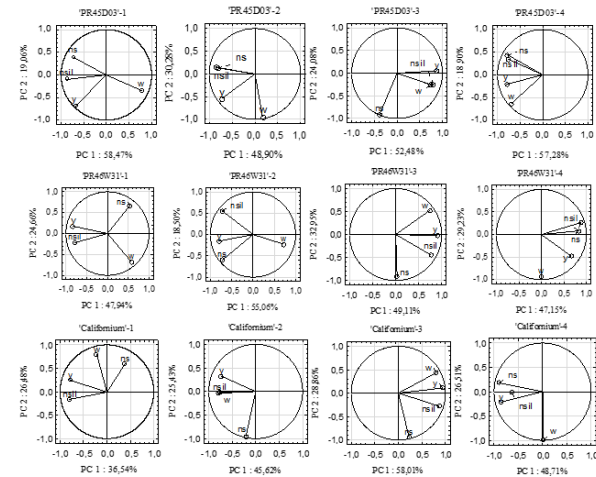


Figure 2. Principal component analysis of individual sowing dates in analyzed cultivar types

Note: (y – yield; nsil - number of siliques per plant ($\text{pc} \cdot \text{m}^{-2}$); ns - number of seeds per silique (pc.); w - weight of one thousand seeds (g); 1-August 14, 2-August 25, 3-September 4, 4- September 15)

Yields in the open-pollinated cultivar were also positively correlated with the number of siliques per m^2 were solely correlated with the number of seeds per silique on the last sowing date. Yields in that cultivar was also significantly positively correlated with the 1000 seed weight on August 25 and September 4. The dependence established using a regression analysis between seed yield and sowing date was parabolic (Figure 3). Our analysis predicts that sowing cv. ‘PR45D03’ 9.3 days after the early sowing date, August 14, would maximize its yields and produce $48.3\text{ dt} \cdot \text{ha}^{-1}$. Sowing the traditional hybrid, ‘PR46W31’, 6.9 days after August 14 (August 22) would maximize its yield and produce $54.8\text{ dt} \cdot \text{ha}^{-1}$. Sowing the open-pollinated cv, ‘Californium’, 7.5 days after August 14 would maximize its yields and produce $49.8\text{ dt} \cdot \text{ha}^{-1}$.

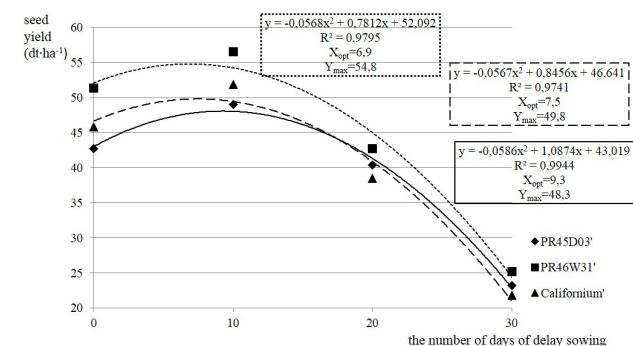


Figure 3. Seed yield ($\text{dt} \cdot \text{ha}^{-1}$) of analyzed cultivars depending on sowing date.

4. Discussion

Recent publications [7, 13] have emphasized the importance of choosing optimal sowing dates to maximize winter rape seed yields while limiting the risk of culture failure [3]. Many authors have stressed that late sowing dates reduce the number of siliques per plant [2], reduce plant height, reduce the number of seeds per silique [14], reduce the number of tillers per plant [15], and reduce seed yield and oil quality [8]. Sowing 10 days earlier relative to the optimal date for the specific climatic and soil conditions of the experiments increased the number of siliques per plant but did not affect other yield components. It should be stressed that sowing rape at the optimal date increased the number of yielding plants and the thousand seed weight, which in turn determined yielding.

We found that traditional hybrids produced significantly more siliques per plant and had greater seed fill per silique than the open-pollinated cultivar, 'Californium', but the 1000 seed weight in the hybrid cultivars was lower than in open-pollinated cultivar. These findings are in agreement with previous studies by Jankowski and Budzyński [9] and Kotecki et al. [10]. In contrast to the assumption of Seeger and Filser [17], the cultivar Artus, a hybrid with the traditional habit, formed significantly more seeds, 749.7 seeds per plant, than the cultivar 'PR45D03'.

Despite considerable breeding progress, both the new and old cultivars exhibit similar responses to sowing dates. All cultivated genotypes, i.e. open-pollinated, complex hybrid, and restoration, exhibited the same trend and scope of response to a delay in sowing date, despite the morphologically differences observed among plants in the autumn [6]. Jankowski and Budzyński [9] also found no differences in the responses among open-pollinated, complex hybrid, and restoration cultivars to delayed sowing.

Plant densities of 60–70 plants·m⁻² are generally considered to be optimal for cultivating rapeseed hybrids in Europe [16], and Wielebski and Wójtowicz [20] recommended an optimal sowing rate, irrespective of the cultivar, of 80 seeds·m⁻², and a reduced sowing rate contributed to an increased number of siliques per plant and a higher 1000 seed weight. Our study confirmed that oil and protein yield did not differ significantly among a seeding rates of 40, 50, and 60 seeds·m⁻², and this finding is consistent with Kwiatkowski et al. [11], who claimed reducing the seed sowing rate to 40 seeds per 1 m² did not increase weed infestation or reduce yield. Dehkordi and Soleymani [5] confirmed that maximum oil yield occurred at a seeding rate of 40 plants·m⁻² and found that increasing the seeding density to 80 and 120 plants·m⁻² caused significant reductions in oil yield, equal to 28.2 and 16.5 kg·ha⁻¹, respectively.

5. Conclusion

(1) The optimal sowing date for all the tested oil-seed rape cultivars was August 25. The hybrid cultivar 'PR46W31' sown on that date exhibited the highest protein and oil yields, and in these respects outproduced cv. 'PR45D03' by 221.6 and 259.2 kg·ha⁻¹, respectively, and outproduced the open-pollinated cv. 'Californium' by 130.9 and 196.9 kg·ha⁻¹, respectively. The regression analysis indicates that the cultivars 'PR46W31', 'Californium', and 'PR45D03' can be expected to produce maximized yields when sown on August 22, August 23, and August 24, respectively.

(2) The semi-dwarf hybrid showed a greater tolerance to delayed sowing compared to the open-pollinated cultivar, and this was confirmed by the dependencies of the main yield components. This cultivar was the only tested cultivar in which yield was strongly correlated with the number of siliques per m², even for plants sown later than the optimal date.

(3) The yields of protein and oil did not differ significantly among plants seeded at densities of 40, 50, 60 pc·m⁻².

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ARTICLE

Contribution of Elemental Sulfur to Soil Acidification, Iron Release and Uptake by Corn (*Zea mays* L.)

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ABSTRACT

A glasshouse experiment was conducted to determine the effects of elemental sulfur (S) applications on soil acidity, the solubility of soil iron, and the uptake of iron (Fe) by corn (*Zea mays* L.). Soil samples were treated with four rates of sulfur and incubated for 0, 20, and 40 days before corn plantation. While one unit increase in S application rate corresponded to a soil pH decrease of approximately 1.52 units, the solubility of the Fe was significantly increased. Fe concentrations in leaves and stems increased as soil pH decreased from 7.03 to 5.42, but further soil acidification decreased Fe concentrations in plant tissues. Overall, applying S at a rate of 0.5 g S kg⁻¹ soil may enhance corn performance by 45 percent while posing minimal risk to groundwater or crops.

1. Introduction

Micronutrient availability in soils depends on soil chemical factors, such as soil acidity and mineralogy^[1, 2]. Iron deficiencies in plants generally occur in calcareous and alkaline soils, in which high pH depresses the solubility of iron (Fe) and zinc (Zn) and decreases nutrient uptake by plant roots^[3]. Applying elemental sulfur to these soils can increase iron solubility and uptake but may also acidify the soil rhizosphere. For every pH unit decrease, there is a corresponding increase in the solubility of Fe and Zn by a factor of 10 to 1000^[4]. Elemental sulfur is especially useful as a soil amendment because it is readily available and acidulates at a gradual rate^[5]. Elemental sulfur acidulates soil as microbial and chemical oxidation convert it to sulfuric acid over time^[6]. It has been reported that applying sulfur above adequate concentrations may improve iron use effi-

ciency in wheat^[7].

Applications of elemental sulfur are likely to improve yields in crops grown under severe iron limitations. It was found that high elemental sulfur supply increased the Fe concentrations in shoots, and that concentrations of iron and sulfur in leaves were significantly correlated, irrespective of iron availability in nutrient solution. Increased sulfur availability may stimulate iron accumulation via increases in methionine, which in turn facilitates the production of elevated levels of phytosiderophores and nicotianamine^[7].

There are contrasting reports on the effects of elemental sulfur applications on soil pH and nutrient availability^[8-10]. As previously noted, applications of elemental sulfur have been found to decrease soil pH and increase nutrient solubility^[14, 15, 16], while some studies have found that nutrient solubility decreased in acidified soils^[11-13]. The increased release

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of soil nutrients from unavailable to available pools could be due to soil pH changes as reported by Ye et al. (2011) and Ye et al. (2010) ^[15, 16]. They also showed that plant nutrient availability increased due to soil pH reductions caused of S applications, and that the mobile fraction of soil heavy metals increased in more acidic soils. The displacement of cations from the exchange complex on clay minerals and soil organic matter occurs via hydrogen ions, and the ion solubility and oxidation state are strongly affected by soil acidity ^[17].

Because soils vary in their responses to elemental sulfur applications ^[18], it is necessary to find an optimal application rate for each one. While the general effects of elemental sulfur applications on soil micronutrient release was elucidated by Karimizarchi et al. (2016) ^[19], the specific impacts of elemental sulfur applications on soil acidity, iron solubility, and iron uptake by plants in the Bintang Series soils have not been studied. Our objective is to quantify the effects of elemental sulfur on the uptake and distribution of iron in corn (*Zea mays* L.) growing in acidified Bintang Series soil.

2. Material and Methods

The Bintang Series soil was amended with 0.0, 0.5, 1.0 and 2.0 g S kg⁻¹ soil and incubated for 0, 20 and 40 days before corn plantation. The corn plants were grown for 45 days under glasshouse conditions. Soil samples were retrieved at the planting and harvesting stages and were subjected to nutrient analysis. In addition, corn leaves, stems and roots were analyzed separately to determine the nutrient content of each part.

2.1 Soil Characterization and Site Description

Soil samples were collected from the surface horizon (0-20 cm) of Bintang Series soil. Collections were made in Bukit Bintang, Perlis, Malaysia (6° 31' 01.61" N, 100° 10' 12.43" E). The collection area was under natural vegetation and its soils were derived from limestone parent materials (Karimizarchi et al., 2014a). The soil samples were air-dried, ground to a particle size of < 2 mm, and shaken for 30 minutes on a reciprocal shaker. The samples were then rested for 24 hours before soil pH and electrical conductivity were measured in a soil-water suspensions (10 g soil to 25 ml deionized water).

2.2 Plant Growth and Management

Seeds of the sweet corn cultivar Masmadu were provided by the Malaysian Agricultural and Development Research Institute ^[19]. Seeds were germinated in laboratory conditions and after 24 hours were transplanted into plastic pots 30 cm in diameter and 50 cm in height. Each pot contained 10 kg of Bintang Series soil and received three plants. After one week the plants were thinned to one. Seedlings were grown for 45

days in a greenhouse located at the University Putra Malaysia (UPM). Each pot was weighed every day, and the soil moisture content was adjusted to 90 percent of field capacity. The plants were irrigated daily. Chemical fertilizers were applied at rates recommended by MARDI ^[20], including 40 kg K₂O per ha in the form of muriate of potash, 60 kg P₂O₅ per ha in the form of triple superphosphate, and 120 kg N per ha in the form of urea.

2.3 Soil and Plant nutrient Extraction and Determination

Since buffered extractants may interfere with the effect of sulfur on nutrient solubility, the available fraction of soil nutrients were extracted using calcium chloride ^[16, 21]. Soil solutions were centrifuged for approximately 15 minutes at 3000 rpm and were then filtered. Plant parts, including root, shoot and leaf tissues, were separately washed with deionized water, dried at 65 °C, and weighed. The tissues were then ground, ashed in a muffle furnace at 480 °C for 10 hours, and dissolved in diluted acid ^[21]. ICP-OES (Perkin Elmer, Optima 8300) was used to determine nutrient concentrations.

2.4 Statistical Analysis

The relationships between soil properties and plant traits were analyzed using various regression models ($p = 0.05$) in Sigmaplot software. Differences among treatments were analyzed using ANOVAs in SAS 9.1, and Duncan's test at $\alpha = 0.05$ was used to identify significance differences among treatment means.

3. Results and Discussion

Soil pH was affected by both the rates and timing sulfur applications (Table 1). For example, the pH values of soil samples incubated for 40 days after being amended with sulfur at rates of 0.5, 1.0, and 2.0 g sulfur per kg soil decreased a background value of 7.51 to 6.66, 5.45 and 4.80, respectively. Plant growth stages also had a significant effect on soil pH (Table 1).

Table 1. Effect of elemental Sulfur application rates (G S kg⁻¹ soil) and timing on soil pH

Sulfur rate	Soil pH							
	At planting				At harvest			
	0	20	40	Mean	0	20	40	Mean
0	7.51Aa	7.44Aab	7.42Ab	7.45Aa	6.99Aa	6.92Aa	6.88Aa	6.93Ab
0.5	7.26Ba	6.75Bb	6.66Bb	6.89Ba	6.30Ba	6.23Ba	6.34Ba	6.29Bb
1	7.22Ca	6.27Cb	5.45Cc	6.31Ca	5.35Ca	5.27Ca	5.17Ca	5.26Cb
2	7.34Ca	5.44Db	4.80Db	5.86Da	3.90Db	3.86Db	4.06Da	3.94Db
Mean	7.33Aa	6.48Ab	6.08Ac		5.63Ba	5.57Ba	5.61Ba	

Averaged across time, soil pH for sulfur application rates of 0.0, 0.5, 1.0 and 2.0 g kg⁻¹ soil decreased from 7.45, 6.89, 6.31 and 5.86 at planting to 6.93, 6.29, 5.26 and 3.94 at harvest, respectively. The relationships of soil pH to incubation time and growth stage suggest oxidation of elemental sulfur occurs gradually and that an incubation time of 20 days was not enough for time the elemental sulfur to oxidize completely. There was no significant difference in soil pH among incubation times for all sulfur application rates at harvest (Table 1). This indicates that the elemental sulfur had been totally oxidized to sulphate at harvest under conditions of this experiment.

The relationship between sulfur rate and soil pH was modelled in order to predict the effects of elemental sulfur additions to Bintang Series soils (Figure 1). The relationship between soil pH and sulfur application rate was linear at the time of harvest, when soil pH values ranged from 6.94 to 3.94. For each unit increase in the rate of elemental sulfur application, the soil pH decreased by approximately 1.52 units. When averaged across three sample dates, the soil pH values were 7.03, 6.29, 5.26 and 3.94 for sulfur application rates of 0.0, 0.5, 1.0 and 2.0 g S kg⁻¹ soil, respectively. The relationship between S application rates and soil pH change is of special interest and needs to be studied for each specific soil. A previous study found that the relationship between S application rate and soil pH was best described using an exponential model, in which applications of 4 tons of S ha⁻¹ decreased soil pH from 7.0 to 4.8, while applications of 12 tons of S ha⁻¹ further decreased soil pH to only 4.2 [22].

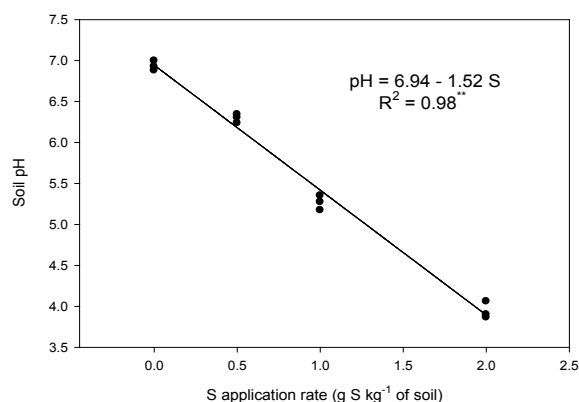


Figure 1. Soil pH changes in response to elemental sulfur application rate

Sulfur applications decreased soil pH and may affect the release of soil Fe, so the correlation between soil nutrient availability and soil pH was studied. A significant correlation of -0.60** was found between soil pH and soil iron. Decreasing soil pH increases the release of soil Fe. This agrees with the general consensus on the positive effect of soil acidification on soil Fe solubility [23, 24, 25].

3.1 Soil acidity and Fe Solubility

The bioleaching of soil nutrients as a function of elemental sulfur application rate and timing in Bintang Series soil was elucidated (Table 2). Additionally, since the acidity produced by oxidation of elemental sulfur in soil is known to increase the solubility of micronutrients [26], the relationship between soil pH and Fe release for Bintang Series soil was quantified (Figure 2). There is no significant change in extractable Fe due to incubation days at planting at each sulfur rate (Table 2). Application of elemental S at 1 and 2 g kg⁻¹ significantly increased extractable Fe only at incubation days of 20 and 40. For instance the concentration of Fe at 40 days of incubation significantly increased from 0.11 mg kg⁻¹ in unamended soil to 0.21 and 0.24 mg kg⁻¹ in soils treated with 1 and 2 g S kg⁻¹ soil, respectively. The extractability of Fe also was significantly affected by growth stage. For instance, averaged across timing, the concentration of Fe increased around 4 times from planting to harvest for highest sulfur application rate. The efficacy of elemental sulfur applications to reduce soil pH and increase soil Fe availability has been previously documented [26]. Our data are in agreement with these results and showed that applications of elemental sulfur decreased soil pH (Table 1). At the same time, decreasing soil pH from 7 to 5 only slightly affected the soil Fe concentration. However, under the conditions of our experiment, further pH reductions increased Fe solubility in Bintang Series soil (Figure 2). This is in line with the observations of other researchers [23], who reported low soil Fe solubility even under very acidic conditions [23].

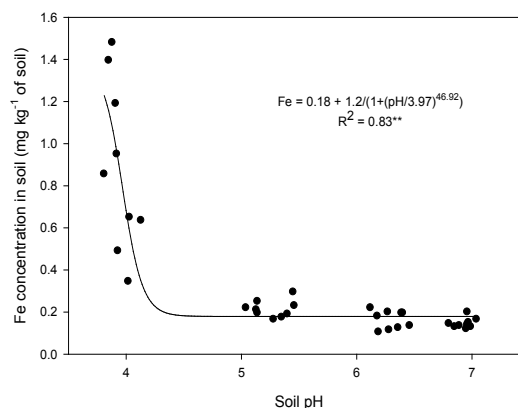


Figure 2. Soil Fe concentration as a function of soil pH

Our data was fitted with non-linear regression model, in which Fe Concentration = $0.18 + 1.2 / (1 + (pH/3.97)^{46.92})$, $R^2 = 0.83^{**}$. The relationship between -Log (Fe Concentration) and soil acidity was fitted with a linear regression model, in which $pFe = 0.25 \text{ pH} - 0.78$, $R^2 = 0.75^{**}$. Although this function is similar to the stability diagrams

for Fe as function of pH that were developed by Khan and Mazid (2011) ^[24], in which $\text{Log Fe}^{2+} = 15.75 - (\text{pe} + \text{pH}) - 2\text{pH}$. However, we found that each unit decrease in soil pH corresponded to a 0.25 unit increase in $\text{Log}(\text{Fe Concentration})$, while Lindsay ^[23] found the each unit decrease in soil pH corresponded to a 2 unit increase in $\text{Log}(\text{Fe Concentration})$. This difference in the observed rate of Fe concentration change due to soil pH reduction can be attributed to differences in soil properties, as well as the assumptions that were considered, between the two studies.

3.2 Fe as an Immobile Nutrient in Corn

Our data showed that the relationship between elemental sulfur application rates and leaf Fe concentrations followed a non-linear quadratic regression model ($Y = 61.5 + 12.58 X - 5.85 X^2$ $R^2=0.67^*$). While increasing elemental sulfur application rates to 2 g S kg⁻¹ soil increased soil Fe concentrations (Table 2), leaf Fe concentrations increased at an elemental sulfur application rate of 1 g S kg⁻¹ soil and decreased at an elemental sulfur application rate of 2 g S kg⁻¹ (Figure 3). The same trend was found for Fe concentration in the corn stem and root (Figure 3). This reflects the fact that corn actively but not passively controls Fe absorption from the soil solution. Our finding is in line with the Marschner (2012) ^[1], who stated that both passive and active mechanisms function in Fe transport across plant cell membranes. In addition, there were differences in Fe concentration in different parts of the corn plants. While the Fe concentrations in roots ranged from 500 to 1500 mg kg⁻¹, Fe concentrations in stem and leaves ranged from 30 to 42 and 59 to 69 mg kg⁻¹, respectively (Figure 3). This observation is in agreement with the results of Barker and Pilbeam (2007) ^[25], who reported that Fe is an immobile element in corn. The highest concentration of leaf Fe, 69 mg kg⁻¹, occurred in leaves at the two intermediate sulfur application rates, and a comparison of Fe concentrations in our corn plants to shoot values (50-300 mg kg⁻¹) ^[25, 1] accepted as adequate shows that Fe was not toxic under the conditions of our experiment.

Table 2. Soil Fe changes in response to elemental Sulfur timing (0, 20, and 40 days application before planting) and application rates (g S kg⁻¹ soil) at planting and at harvest.

Sulfur rate	Soil Fe (mg kg ⁻¹ soil)							
	At planting				At harvest			
	0	20	40	Mean	0	20	40	Mean
0	0.21Aa	0.14BCa	0.11Ba	0.15BCa	0.14Ba	0.16Ba	0.13Ba	0.14Ba
0.5	0.14Aa	0.12Ca	0.09Ba	0.12Cb	0.17Ba	0.17Ba	0.14Ba	0.16Ba
1	0.12Aa	0.18Ba	0.21Aa	0.18ABa	0.25Ba	0.18Bb	0.20Bb	0.21Ba
2	0.15Aa	0.25Aa	0.24Aa	0.21Ab	0.94Aab	1.17Aa	0.54Ab	0.88Aa

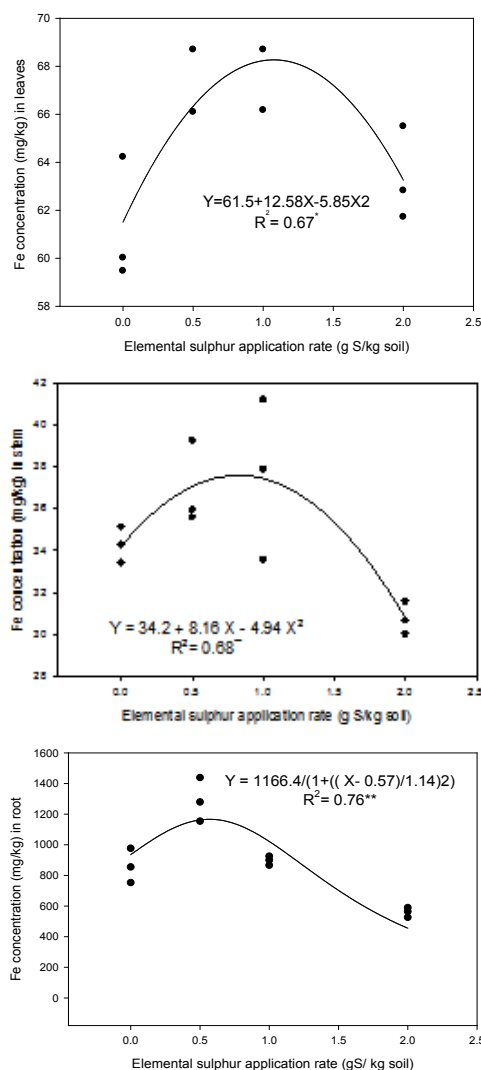


Figure 3. Relationship of elemental sulfur application rate and iron concentration in different parts of corn

3.3 Corn Performance as a Function of Fe Concentration

There was no significant relationship between leaf dry weight and leaf Fe concentration. Leaf Fe concentrations ranged from 60 to 75 mg kg⁻¹ (Figure 3), while the recommended range of corn leaf Fe concentrations range from 50 to 300 mg kg⁻¹ ^[1]. However, there is a significant relationship, $Y = 19.3 / (1 + ((X - 37.6) / 5.4)^2)$, $R^2 = 0.695^{**}$, between Fe concentrations in stem and stem dry weight. According to this model, the maximum performance of corn, 19.3 g pot⁻¹, was obtained at Fe concentrations of 37.6 mg kg⁻¹ in the stem (Figure 4). Because maximum corn production, in terms of all leaves, stem and root weight, was not obtained at maximum Fe content, it can be concluded that the Fe was not the main limiting factor for corn growth under the conditions of our experiment.

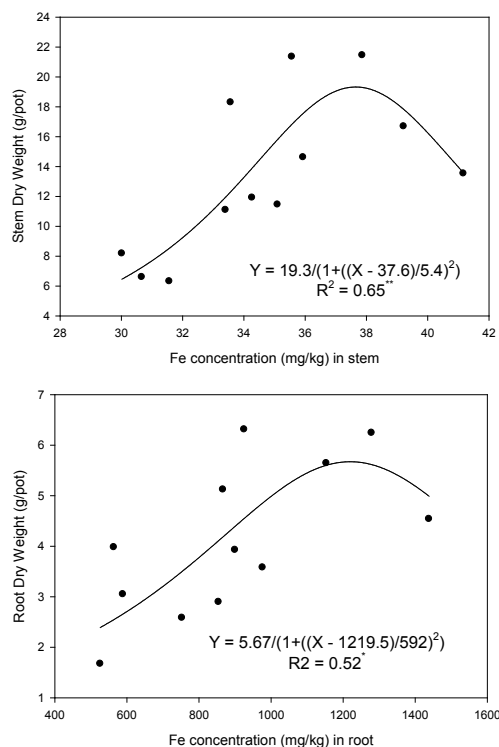


Figure 4. Relationship between Fe concentration in corn and corn performance

4. Conclusion

As soil pH decreased by 1.52 units with each unit addition of elemental sulfur, it can be concluded that elemental sulfur is an appropriate means for lowering pH in Bintang Series soils. In addition, our results demonstrated that extractable Fe was not significantly affected by soil acidification due to elemental sulfur application until soil pH decreased to approximately 4, at which point hydrous oxide precipitates, and applications of 2 g S kg⁻¹ soil increased the CaCl₂ extractable Fe by 5.57 times. Moreover, our results showed that this increases in soil Fe solubility failed to increase Fe concentration in corn leaves, stem and root. In conclusion, applying of elemental sulfur at a rate of 0.5 g S kg⁻¹ soil is recommended to improve corn performance by 45 percent while posing minimal risks to corn production and groundwater quality.

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ARTICLE

Comparative Toxicity of Neem and Peppermint Oils Nano Formulations against *Agrotis ipsilon* (Hufn.) Larvae (Lepidoptera: Noctuidae)

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ABSTRACT

Applications of nanotechnology in agriculture will result in the development of efficient and potential approaches towards the management of insect pests. The toxicity effects of four essential oils peppermint, thyme, camphor and sage oils were tested against the fourth instar larvae of *Agrotis ipsilon* to select the most effective essential oil to be converted to the nano form. According to the results obtained, peppermint oil was the most toxic compound, which has been used in the present investigation compared with neem oil. The toxicity of bulk and nano- formulations of neem and pepper mint oils were tested against 2nd and 4th instar larvae of *A. ipsilon* under laboratory conditions of 25 ± 2 °C & 65 -70 % R.H. relative humidity. The results show that the LC50 value (the concentration used which kill 50% of the tested individuals) of loaded neem or pepper mint were lower (0.62 and 36.47 ppm) compared with neem or pepper mint oil nano-emulsion and bulk neem for the second larval instar. The different formulations of neem are more potent than in case of peppermint oil, as LC50 and LC90 values were significantly lower.

The same trend was found concerning the 4th larval instar. Age of treated larvae had a detrimental effect on the response to the compounds tested. It was noticed that the younger larvae were much more sensitive to the prepared compounds compared to the older ones. The least LC50 value for loaded neem nano-emulsion was 6.68 ppm compared with the highest value for bulk neem oil (16.68 ppm). Also, LC90 values followed the same trend as in case of LC50. Again, the toxicity of loaded peppermint oil had the most insecticidal activity as expressed by the lowest LC50 value (51.9 ppm) with more insecticidal effect than the bulk (125.43 ppm) or nano-emulsion (85.43 ppm).

The present results indicated that these novel systems could be used in integrated pest management program for *A. ipsilon* control.

1. Introduction

Black cutworm (*Agrotis ipsilon* Hufnagel) is a cosmopolitan pest that affects over 30 important crops, including beans, broccoli, cabbage, carrot,

spinach, eggplant, lettuce, potato, tomato, and turnip^[1, 2]. Black cutworm larvae feed above ground, and each one can consume over 400 cm² of foliage during its development. The terminal and subterminal instar stages account for 80% and 10% of foliage, respectively, and minimal fo-

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liage loss occurs during the early stages of development. Larvae in the fourth instar stage may sever the stems of young plants, and one larva may sever the stems of several plants in a single night^[4].

Insecticides currently used to control *A. ipsilon* infestations are hazardous to humans and animals. Insecticides derived from plants may be a safer alternative^[6] and could be used as a cost-effective means of pest control for farmers in developing countries if simple extracts can be prepared from readily available plants^[5].

Neem (*Azadirachta indica* A. Juss.) is a limonoid-producing species in the mahogany family (Meliaceae Juss.) and has long been recognized as a source of environment-friendly biopesticides. Extracts from its leaves and seeds act as antifeedants, repellants, and growth disruptors in multiple insect species^[8, 9, 10]. They are, however, relatively selective, and neem products have been recommended for many Integrated Pest Management programs^[7].

Sharaby and El-Nojiban^[11] studied the toxicity of different essential oils against the greasy cut worm. They also added that all tested oils have great effect on growth and development of *A. ipsilon* larvae. Kamaraj et al^[12] showed reduction in growth and development of *H. armigera* and *S. litura* larvae when fed exclusively on Neem gum nano formulation (NGNF) treated castor leaves.

Although, biotechnological advances existed when botanical material used in plant protection, the major points have been taken in consideration are that of a rapidly degradation and polluting the ecosystem caused by showed prevailing practices. To take the situation it was need to harness innovative approaches towards other solutions such as nanotechnology^[13]. Pesticides in nanoparticulate form present an attractive solution for this problem. Their effective concentration is expected to be much lower compared to that of bulk materials and they can be formulated without the use of organic solvents. Sodium alginate (Na-Alg) has been used as a controlled release matrix material in medicine^[14] and agriculture^[15] after cross linking it with calcium chloride and glutaraldehyde. Alginate polysaccharides are identified to be hemocompatible and do not build up in any organs of the human body. Encapsulating nano-particle layers at the emulsion droplet interface may be engineered to increase droplet stability and control of release kinetics. Based on the for mentioned facts, The present investigation aimed to study the effects of three different formulations, bulk, nano-emulsion and loaded nano-emulsion of two main botanical extract, i.e. neem extract and peppermint essential oil against *Agrotis ipsilon* larvae.

2. Materials and Methods

2.1 Test Insect

Black cutworms *Agrotis ipsilon* (Hufn) were maintained for several generations in rearing units under controlled conditions of $25 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. The adult moths were reared in glass jars measuring 15x25cm. A sucrose solution of 10% concentration was provided for feeding the moths. Females laid their egg masses on black muslins, newly-laid egg masses were collected. The old muslins were replaced by new ones, and the adult moths were provided with fresh feeding solution.

The newly laid eggs were categorized according to their oviposition date and were immediately placed in a suitable container. A small hair brush was used to transfer newly hatched larvae into plastic boxes measuring 25x15cm and containing a suitable amount of clean castor leaves. Larvae at the third instar were separated into individual plastic boxes to prevent cannibalism. Pupae were collected from the larval containers and were transferred to containers of sawdust.

2.2 Tested Plant Extract and Essential Oils

Neem, peppermint (*Mentha x piprera* L.), camphor (*Cinnamomum camphora* (L.) J. Presl.), thyme (*Thymus vulgaris* L.), and sage (*Salvia officinalis* L.) oils were investigated. Neem oil (0.03% azadirachtin) derived from neem seed kernel extract was obtained from Dr. Kleeberg in Lahnaw, Germany. Peppermint oil with a menthol content of 26% was derived from peppermint (*Mentha x piprera* L.) using the method described by Guenther (1961)^[16]. The remaining essential oils were obtained from the 1 Oil extraction unit at the National Research Centre in Cairo, Egypt.

3. Preliminary Screening Tests of Essential Oils against *A. ipsilon* Larvae

Bioassays were carried out using peppermint, thyme, camphor, and sage essential oils on fourth instar *A. ipsilon* larvae, and the most effective oil was used for the present investigation. Larvae were fed 100g of a semi-synthetic diet as described in Shorey and Hale (1965)^[17]. The diet was prepared using 500 g kidney beans, 30 g agar, 65g yeast, 3g sorbic acid, 5 g benzoic acid, 10ml formalin, and 10 g ascorbic acid. The kidney beans and agar were autoclaved in 600ml of distilled water and were then ground with the other components, except ascorbic acid, which incorporated with the prepared media after it had cooled to the appropriate temperature. Each essential oil was included in a series of increasing

concentrations in order to calculate LC_{50} values. Concentrations of 0.12, 0.25, 0.5 and 1% were incorporated as aqueous dilutions into 100 g of the semi-synthetic diet. This procedure was carried out immediately before gelling in order to avoid decomposition. Media treated with distilled water and a 100 μ l of Tween 80 was used as control. The selected larvae were tested using four replicates per concentration per essential oil with ten larvae in each replicate. Each replicate was housed in a glass tube 10 cm in length and was fed 1 g of the treated diet.

The larvae were incubated at 25 ± 2 °C and 65-70% RH. Larval mortality was recorded daily for 4 days after treatment and compared with the control larvae. The mortality percentage was corrected using Abbott's formula^[18]. The logarithmic (?) relationship between the oil concentrations and larvae mortality were plotted and the LC_{50} values were calculated using Ld-p line program according to Finney (1971)^[19].

The results of the primary screening tests were used to select peppermint oil, in addition to the neem extract, for continued experimental work.

4. Preparation of Nano-formulations

4.1 Nano-emulsions Preparation

Emulsions of neem or peppermint oil, Tween 80, and distilled water were prepared using a modification of the method described by Jerobin et al. (2012)^[20]. Neem oil was diluted with distilled water to a ratio of 1:1 (oil to water), and peppermint oil was diluted with distilled water to a ratio of 2: 1 (oil to water). Two percent of Tween 80 was added as an emulsifier. The emulsion was then sonicated for 30 minutes using an ultrasonic cleaner set (model WUC-DO3H 290W) set at 60 Hz. It was then resonicated for 1 minute using a high energy ultrasonication probe (model VCX750) set to 750W and 20 kHz, and it was then resonicated again for 30 minute by the ultrasonic cleaner set under cooling conditions^[21].

4.2 Preparation of Loaded Nano-emulsions

Alginate nanocapsules were prepared using oil in water (o/w) emulsifications, followed by cross linking using calcium chloride and solvent removal (in case of using solvent), using modified versions of the methods described by^[22, 23]. Sodium alginate solution (3%, w/v) was prepared by dissolution in distilled water at 50°C for 45 min. Tested oils were diluted by distilled water using Tween 80 as an emulsifier with mechanical stirring for 10 min. Briefly, sodium alginate o/w emulsion was made by drop wise dispersion of diluted oil into appropriate

volume of alginate solution (1 : 1 oil to alginate in case of neem oil and 1 : 2 in case of pepper mint oil under continuous mechanical stirring at room temperature. The emulsion thus formed were sonicated for 30 min using ultrasonic cleaner set, model WUC-DO3H 290 W and 60 Hz and then sonicated for 2min using a high energyultra sonication probe model VCX 750, 750 W, 20 kHz). An appropriate volume of $CaCl_2$ (2:10 $CaCl_2$ to alginate, respectively) was then added into the resulting emulsion and stirred for an additional 30 min and sonicated as mentioned previews. Nano- capsules were obtained as dispersion in aqueous solution

In another experiment, pepper mint oil was tested with diluting by ethanol as a solvent instead of water and was removed after equilibration under reduced pressure at 40–45°C for 20 min.

5. Bioassay Tests

Bioassays tests were carried out on second and fourth instar *A. ipsilon* larvae. Samples of 100g semi-synthetic diet previously described by^[17] were treated. All prepared formulations were incorporated into the diet as aqueous dilutions at the desired concentrations during the preparation of the diet. Series of concentrations of each formulation were used to calculate the LC_{50} values. Such procedure was carried out just before gelling in order to avoid decomposition of the used materials. Media treated with distilled water mixed with 100 μ l of Tween 80 was used as control. All concentrations were prepared according to the active ingredient content in each formulation.

In case of 2nd instar larvae, 5g of treated semi-synthetic diet for each concentration was added in plastic cups of 120ml in capacity. Serial concentrations of bulk neem oil containing 1.5, 3, 6, 9 and 12 ppm azadirachtin were prepared. Neem oil nano-emulsion and loaded neem oil nano- emulsion were prepared by concentrations of 0.75, 1.5, 3 and 6 ppm azadirachtin. Ten 2nd instar larvae were then transferred to each cup with four replicates, of 10 larvae /replicate.

Bioassay tests of pepper mint oil were carried out using serial concentrations of pepper mint oil containing 30, 60, 90 and 120 ppm menthol for bulk oil. For nano-emulsion and loaded nano- emulsion formulations, serial concentrations of 5, 10, 20, 30, and 40 ppm were added to the targeted medium. Ten of 2nd instar larvae were then transferred to glass tubes with four replicates, of 10 larvae /replicate.

In case of 4th instars larvae, glass tubes of 10 cm height were used. One piece weighted 1g of treated diet was cut by the cork borer and was added in each glass

tube and each larva was transferred individually to each tube with four replicates, of 10 larvae /replicate. Bulk neem oil was tested in concentrations of 6, 12, 18 and 24 ppm azadirachtin. Neem nano- emulsion and loaded nano-emulsion were tested in concentrations of 3, 4.5, 6, 7.5 and 9 ppm. Bulk pepper mint oil was prepared in the concentrations of 30, 60, 120 and 280 ppm menthol. Nano- emulsion and loaded nano- emulsion were prepared in concentrations of 30, 60, 90 and 120 ppm menthol.

Those cups and glass tubes were incubated at 25 ± 2 °C and 65 -70% R.H. Larval mortality was recorded daily during 4 days after treatment and adjusted for control experiment and the mortality percentage was corrected using Abbott's formula^[18]. Concentrations mortality regression lines were plotted in form of log/probit relation and the LC₅₀ values were calculated using Ld-p line program according to^[19].

6. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) using SPSS software (Tukey test). A value of $p < 0.05$ was considered statistically significant.

7. Results and Discussion

7.1 Preliminary Bioassay Tests of Essential Oils against *A. ipsilon* Larvae

In order to determine the most effective essential oil to be converted to the nano form, the toxicity effect of four essential oils Pepper mint, Thymus, Camphor, and Sage oil were tested against the 4th instars larvae of *A. ipsilon*. Their toxicity against the 4th instar larvae are given in Table 1. It was shown that pepper mint was the most effective essential oil and thymus oil was the least effective oil. LC₅₀ values were 0.45%, 0.60%, 0.73% and 0.86 % for pepper mint, camphor, sage and thyme, respectively after 96hr post treatment. The toxicity index values were 100, 75.00, 61.64 and 52.33%, respectively. The relative potency values were 1.91, 1.43, 1.18 and 1, respectively. The present findings agreed with that recorded by^[11]. They arranged the toxicity values of some oils based on LC₅₀ values tested against *A. ipsilon* in descending orders as follows garlic, mint, Cumin, caraway and parsley. As shown the second toxic essential oil was the mint oil. They added that LC₅₀ value of mint oil was 0.032% as a contact poison on the larvae. LC₅₀ for larvae as a stomach poison and on pupal stage nearly equal they were 0.160 and 0.148%, respectively.

Table 1. LC₅₀ and LC₉₀ values of different essential oils on *A. ipsilon* 4th instars larvae after 96h post treatment

Essential Oils	LC ₅₀ ml (Fudicial limits)	LC ₉₀ ml (Fudicial limits)	Slope	Regression	Toxicity index	Relative potency
Pepper mint	0.45 (0.37-0.54)	1.20 (0.91-1.86)	3.01±0.41	0.99	100	1.91
Thyme	0.86 (0.73-1.05)	2.34 (1.76-3.69)	2.96±0.40	0.98	52.33	1
Camphor	0.60 (0.50-0.70)	1.44 (1.15-2.05)	3.33±0.42	0.98	75.00	1.43
Sage	0.73 (0.60-0.93)	1.87 (1.33-3.44)	3.12±0.50	0.97	61.64	1.18

7.2 Insecticidal Activity of Neem NanoFormulations against the 2nd Instar Larvae of *A. ipsilon*

Data represented in Table 2 reveal that loaded nano-emulsion from neem oil when tested on the 2nd instar larvae had the highest insecticidal activity as expressed by lowest LC₅₀ value while the bulk crude oil presented the highest LC₅₀ value. The LC₅₀ values were 0.62, 2.36 and 4.38 ppm for neem loaded nano-emulsion, nano-emulsion and bulk crude oil, respectively. Also LC₉₀ values behaved the same activity order as they were 4.86, 5.70 and 8.57ppm, respectively. Slope values were 1.43, 3.34 and 4.40 with regression coefficient 0.99, 0.98 and 0.99 and toxicity index 100, 26.27 and 14.16.

7.3 Insecticidal Activity of Pepper Mint Oil Nano Formulations against the 2nd Instar Larvae of *A. ipsilon*

Data in Table 2 show descending order of pepper mint oil nano formulations on the 2nd instar larvae based on their LC₅₀ values as they were bulk oil, nano-emulsion and loaded nano-emulsion represented by 55.77, 43.25 and 36.47 ppm, respectively. LC₉₀ values were 163.58, 79.72 and 107.20 ppm, for the three formulations respectively. The relative potency was 1.53 fold for loaded nano-emulsion and 1.29 fold for nano-emulsion more than bulk oil. Slope values were 2.74, 4.83 and 2.74, for bulk, nano-emulsion and loaded nano-emulsion, respectively. Also, toxicity index values were 65.39, 84.32 and 100 for the same order shown previously. The regression coefficient values were 0.99 for both bulk and nano-emulsion, and was 0.97 for loaded nano-emulsion.

Nano-emulsions of pesticidal active ingredients (AIs) have often been suggested to increase the uptake of the AIs, but supporting data in the context of plant protection products remains scarce^[24]. The same authors reported

that a series of nano-emulsions of neem oil decreased the LC_{50} (the concentration required to achieve 50% mortality) with decreasing of droplet size, which was interpreted as indicating an increased uptake of smaller droplets. In another study, the efficacy of a nano-emulsion of permethrin presented in ^[25] was significantly higher than that of the pure AI, which was again interpreted as indicating an increased uptake of the nano-formulated. In a study mentioned by ^[26], it was shown that the effects on non-target organisms (*i.e.*, soil bacteria and plants) were reduced, but the reasons for the different effects on target and non-target organisms have yet to be elucidated. Unfortunately, no comparisons were carried out with commercial formulations. ^[25] agreed with the present findings that nano-permethrin was more potent in its larvicidal effect against *C. quinquefasciatus* than the bulk form of permethrin. The LC_{50} of nano-permethrin (0.117mg/L) was found to be more effective compared to bulk permethrin (0.715 mg/L). 100% mortality was recorded within 6h for nano permethrin treated samples. But for bulk permethrin treated samples 100% mortality was not observed even after 24h of exposure period.

7.4 Insecticidal Activity of the Different Nano-Formulations from Neem against the 4th Instar Larvae of *A. ipsilon*

Age of treated larvae had a detrimental effect on the response to the compounds tested. It was noticed that the younger larvae were much more sensitive to the prepared compounds compared to the older ones. That was clear in Table 3 showing the LC_{50} values of such preparations on 4th instar larvae of *Agrotis ipsilon*. The least LC_{50} value for loaded neem nano-emulsion represented by 6.68 ppm followed by nano-emulsion that was 10.82 ppm and the highest LC_{50} value was 16.68 ppm for bulk neem oil. Also, LC_{90} values were 10.15, 14.19 and 34.74 ppm. Relative

potency of loaded nano-emulsion was 2.50 fold and nano-emulsion was 1.54 fold more than bulk oil. In addition, slope values were 4.02, 10.89 and 7.01 and regression was 0.91, 0.99 and 0.98 for bulk, nano-emulsion and loaded nano-emulsion, respectively and toxicity index was 40.05, 61.74 and 100, respectively.

7.5 Toxicity Effect of the Different Nano-formulations from PepperMintOil on the 4th Instar Larvae of *A. ipsilon*

Similar but less pronounced toxic effects were recorded in studies carried out with the 4th larval instar. Loaded nano-emulsion from peppermint had maximum insecticidal activity as expressed by the lowest LC_{50} value (51.90 ppm) with more insecticidal effective 2.42 fold than the bulk oil followed by nano-emulsion (85.43 ppm) with relative potency 1.47 fold than the bulk oil which showed the highest LC_{50} value (125.43 ppm) (Table 3). LC_{90} values were 89.75, 253.09 and 334.80 ppm, respectively. Slope values were 5.39, 2.72 and 3.01. Toxicity index were 100, 60.75 and 41.38. Regression values were 0.96, 0.99 and 0.99 for loaded nano-emulsion, nano emulsion and bulk respectively.

^[27] reported that larvicidal and repellent activities of essential oils have been attributed to their major monoterpenic constituents. The rapid action against some pests is indicative of a neurotoxic mode of action related to the monoterpenes substances. Also, there is evidence effect on acetylcholinesterase and octopaminergic system in insects as confirmed by ^[28, 29]. Generally essential oils and their component have been considered safe than other plant derived chemicals like azadirachtin, rotenone or pyrethrum^[30]. This could be attributed to existing detoxifying metabolism pathways and bio rational mode of action of monoterpenoids as reported by ^{[31],[32]} mentioned that the rapid action of essential oils against some pests

Table 2. LC_{50} , LC_{90} of the neem and peppermint oil nano-formulations against 2nd instar larvae of *A. ipsilon*

Tested plant extract or Essential oil	Formulation	LC_{50} (ppm) (Fudicial limits)	LC_{90} (ppm) (Fudicial limits)	Slope	Regression	Toxicity index	Relative Potency
Neem	Bulk	4.38 (3.81 - 5.01)	8.57 (7.21 - 11.05)	4.40 ± 0.56	0.99	14.16	1
	Nano emulsion	2.36 (2.03 - 2.90)	5.70 (4.18 - 10.56)	3.34 ± 0.60	0.98	26.27	1.86
	Loaded nano emulsion	0.62 (0.33 - 0.89)	4.86 (2.91 - 14.93)	1.43 ± 0.31	0.99	100	7.06
Mentol	Bulk	55.77 (46.33 - 69.05)	163.58 (118.21 - 287.21)	2.74 ± 0.42	0.99	65.39	1
	Nano emulsion	43.25 (38.12 - 49.33)	79.72 (67.02-103.85)	4.83 ± 0.63	0.99	84.32	1.29
	Loaded nano emulsion	36.47 (29.72-46.17)	107.20 (77.58 - 177.74)	2.74 ± 0.36	0.97	100	1.53

Notes: LC_{50} and LC_{90} values were calculated and expressed as ppm active ingredient (azadirachtin in neem and menthol in peppermint)

Table 3. LC₅₀, LC₉₀ of the neem and peppermint oil nano-formulations against 4th instar larvae of *A. ipsilon*

Tested plant extract or Essential oil	Formulation	LC ₅₀ (ppm) (Fudicial limits)	LC ₉₀ (ppm) (Fudicial limits)	Slope	Regression	Toxicity index	Relative Potency
Neem	Bulk	16.68 (13.99 - 20.95)	34.74 (25.70 - 76.16)	4.02 ± 0.98	0.91	40.05	1
	Nano emulsion	10.82 (10.18 - 11.46)	14.19 (13.15 - 16.07)	10.89 ± 1.65	0.99	61.74	1.54
	Loaded nano emulsion	6.68 (6.11 - 7.36)	10.15 (8.90 - 12.66)	7.01 ± 1.07	0.98	100	2.50
Mentol	Bulk	125.43 (104.76-152.25)	334.80 (253.81- 520.44)	3.01 ± 0.41	0.99	41.38	1
	Nano emulsion	85.43 (66.20-109.86)	253.09 (177.25 - 510.50)	2.72 ± 0.52	0.99	60.75	1.47
	Loaded nano emulsion	51.90 (44.28 – 60.05)	89.75 (74.89 – 123.94)	5.39 ± 0.97	0.96	100	2.42

Notes: LC₅₀ and LC₉₀ values were calculated and expressed as ppm active ingredient (azadirachtin in neem and menthol in peppermint oil)

is an indication of a neurotoxic mode of action related to monoterpenes substances. Larvicidal and repellent activities of essential oils have been attributed to their major monoterpenic constituents. Also, there is evidence effect on acetylcholinesterase and octopaminergic system in insects as confirmed by^[28, 29]. The obtained results show that different nano formulations of the two tested botanical oils have significant effects on the toxicity of the 2nd and 4th larval instars of *A. ipsilon*. Neem nano-formulations were more potent in its larvicidal effect against *A. ipsilon* than peppermint nano-formulations.

However, more information is needed before these toxic effects can be fully explained and understood in Integrated Pest Management (IPM) programs using this plant extract and essential oils.

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ARTICLE

Diversity and Biomass of Understory Plants in *Larix gmelinii* Forest under Different Reconstruction Methods

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ABSTRACT

The effects of different management measures on the undergrowth diversity of *Larix gmelinii* forests were determined. The undergrowth vegetation of Xing'an Larch forest under seven different transformation methods was investigated in the Chaocha Forest Farm of the Genhe Forestry Bureau in the northern Daxinganling Mountains. Community composition, structural characteristics, species diversity and biomass of seven different retrofitting methods and one control plot. The results showed that the species composition of *Larix gmelinii* under 7 different transformation methods included 34 species of 30 genera and 21 families of shrubs, including 7 species, 7 genera and 7 species of shrub layer, and 20 species, 24 genera and 24 species of common species in the herb layer. The species with the largest proportion in the layer is bilberry, followed by Xing'an rhododendron, and the dominant species of herbaceous layer is quite different. In terms of diversity index, the diversity index of Xing'an larch forest under local tending artificial promotion natural regeneration and transformation measures was low ($P < 0.05$); the study showed that the best tending thinning intensity was between 30% and 40%, different. The impact of the transformation method on the structure and diversity of understory vegetation in *Larix gmelinii* forest is not only related to the transformation, but also depends on the transformation measures taken.

1. Introduction

Dahurian larch (*Larix gmelinii* (Rupr.) Rupr.) forests are mainly distributed in northeastern Asia. Due to long-term over-utilization and poor protection, the Daxinganling natural forest has changed its original structure and function, and lost its natural regularity. Therefore, it is necessary to optimize the structure with different management measures to improve the stability of the ecosystem. Baskin^[1] showed in the experiment that the increase of species diversity can improve the stability of the eco-

system. And the same results were obtained in the study of forest communities^[2-4]. Species diversity is a manifestation of biodiversity at the species level, which can characterize the structural complexity of a biome, reflecting the structural type, organization level, development stage, stability level and habitat level of the community. It is a biodiversity. The important organic component is the research hotspot in the field of ecology^[5]. At the same time, the restoration of species diversity is also one of the most important features in the process of vegetation restoration^[6]. Our the biomass,

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diversity and structure of understory vegetation to reflect the effects of the transformation measures.

1.1 Overview of the Study Area

The test site is located in the experimental area of the National Wildlife Science Research Station, located in the Greater Xing'an Mountains Ecosystem in Inner Mongolia, in the Chaocha Forest Farm of the Genhe Forestry Bureau in the northern part of Daxing'anling. It is a typical cold temperate northern forest area. The highest altitude is 1199 m, the annual average temperature is -5.0°C , the annual average precipitation is 400-500mm, the frost-free period is 80-90 days, and the annual effective accumulated temperature of $\geq 10^{\circ}\text{C}$ is 1308.9°C . The zonal soil is brown coniferous forest soil, grey forest soil, black calcium soil, the bedrock is mainly granite and basalt, the thickness of soil layer is 5 ~ 40 cm, there are continuous permafrost and island permafrost staggered distribution in this area. There are 363 species belonging to 212 genera and 74 families in this area. *L. gmelinii* forest is a cold temperate coniferous forest dominated (?) by *Larix gmelinii*. Associated tree species include *Betula platyphylla* Suk. and *Populus davidiana* Dode., and characteristic understory species include *Ledum palustre* L., *Rhododendron dauricum* L., *Vaccinium uliginosum* Linn., *Pyrola incarnata* Fisch. ex DC.

1.2 Research Methods

1.2.1 Design of Different Renovation Measures

Seven fixed sample plots were set up in *L. gmelinii* and *B. platyphylla* communities in 2012. Each plot was subject-

ed to a different treatment. The first plot was managed to artificially promote the update. A 1 m^2 small quadrant was set up to remove shrubs and herbs in the small quadrant, throw away dead cover layer (dry branches and fallen leaves), expose the surface layer of soil, improve the probability of seed contact with soil, promote seed rooting and germination, and promote natural regeneration of stand. The second plot was managed to promote mixed forest. 1 to 2 *B. platyphylla* with straight trunk shape, full crown and good growth should be kept in each cluster. 2-year-old *L. gmelinii* seedlings with needle insertion were planted in the gap of the sample plot and a density of 2490 plants/ hm^2 , and 398 *B. platyphylla* were planted in the sample plot. The third plot was tending and thinning: thinning the cluster *Betula platyphylla* in the sample plot. The thinning objects are birch grade 4 and 5 trees, upper trees with diameter over 14 cm and overlord trees. Cutting volume intensity was 30%, and plant number intensity was 59%. The fourth plot was tending and intermittent cutting of forest: thinning the cluster birch in the sample plot, keeping 1-2 *Betula platyphylla* with straight trunk shape, full crown and good growth in each cluster. Cutting down dead trees, *P. davidiana* Dode and other non-target species, as well as the overlord trees with diameter over 19 cm at breast height of *B. platyphylla*. Cutting volume intensity was 20%, and number intensity was 13%. V. Local nurturing artificially promotes regeneration: Intermediate cutting of Cluster *B. platyphylla* in sample plot showed that the cutting volume intensity was 15% and the number intensity was 45%. Setting up a 1 m^2 small sample, clearing shrubs and herbs in the small sample, throwing away dead cover layer (dry branches and fallen

Table 1. Existing forest conditions after different transformation methods

Standard place number	Basic situation of forest stand	Business conditions	Existing forest	
			Density/strain· hm^{-2}	Average breast diameter / cm
I	_____	Manual promotion of renewal by abandoning ground cover in 2012	2488	11.6
II	Survival rate of supplementary seedling 77%	Mixed forest was induced by replanting Larch Seedlings in 2012	2781	7.6
III	_____	30% thinning intensity in 2012	2925	10
IV	_____	20% thinning intensity in 2012	4375	10.9
V	_____	Artificial Removal of Ground Cover to Promote Renewal after Local Care in 2012	1106	11.8
VI	_____	40% thinning intensity in 2012	4325	7.7
VII	_____	10% thinning intensity in 2012	2725	10.6
VIII	_____	Through natural sparseness to retain trees, do not operate.	2925	10

leaves), exposing the surface layer of soil, improving the probability of seed contact with soil, promoting seed rooting and germination, and promoting natural regeneration of stand. The sixth plot was treated by intermittent cutting of forest. Young trees and grade 5 trees of *B. platyphylla* were felled and regenerated. Thinning dead trees, *P. davidiana* Dode and other non-target species, as well as the upper layer of *Betula platyphylla* whose DBH is more than 14 cm. Cut down the upper layer and some 4-5 grade trees of *L. gmelinii* whose DBH is more than 14 cm. Cutting volume intensity was 40%, and plant number intensity was 50%. The seventh plot was treated by cutting down dead trees of *P. davidiana* Dode and *B. platyphylla* to regenerate young trees and *Betula platyphylla* overlord trees in the sample plot. Cutting volume intensity was 10% and plant number intensity was 18%. The eight plot acted as a control. The following is a picture of the existing forest land after management and transformation (Table 1.)

1.2.2 Biomass survey

The arbor biomass^[7] of *L. gmelinii* was determined using the equation: $W=0.1359 D^{2.4077}$, $R^2=0.983$. The arbor biomass of *B. platyphylla* was determined using the equation: $W=0.02853(D^2H)0.89278+0.00278(D^2H)^{1.02568}+0.01545(D^2H)^{0.61265}+0.023923(D^2H)^{0.69612}$

The biomasses of the shrub and herb layers were measured using a quadrat harvesting method^[8,9]. The species and quantity of shrubs and herbaceous plants were registered. The area of the shrub layer was 2 m². The aboveground biomass was measured. The area of herbaceous layer was 1 m². The aboveground biomass was measured. The litter biomass was investigated together with the biomass of shrubs and herbaceous layer in the same layer.

1.2.3 Plant Diversity Analysis

Eight plots were determined. Five plots were set in each plot. Density (plant/m²), average crown width (m²/plant), coverage (%), that is, the ratio of the total elliptic area of all tree crowns to the area occupied by the ground), average breast diameter (cm/plant), average height (m/plant), aboveground biomass^[10-16] were measured to calculate community diversity. Diversity measures include:

(1) Richness:

$$R = (S - 1) / \ln(N)$$

Among them, S is the number of species in the community, A is the total number of individuals observed.

(2) Shannon-Wiener diversity index:

$$H' = - \sum_{i=1}^S P_i \ln P_i$$

(3) Pielou index of uniformity:

$$J = H' / \ln S$$

(4) Simpson diversity index:

$$D = 1 - \sum_{i=1}^S P_i^2$$

S is the total number of species in the community, P_i is the proportion of species I in the total number of individuals in the community, that is, P_i = N_i / N, N_i is the number of species i, and N is the total number of observed individuals.

Important value = (relative frequency + relative abundance + relative coverage) / 3

2. Results and Analysis

2.1 Species Composition and Important Values

There are 34 species belonging to 30 genera and 21 families, including 7 species belonging to 7 genera and 3 families of shrubs and 24 species belonging to 24 genera and 20 families of herbs.

Shrub layer: According to the statistics in the table below, there are three species of shrubs: *Vaccinium vitis-idaea* Linn., *Rhododendron dauricum* L., *Rosa bella* Rehd. The endemic species is *Salix raddeana* Laksch. ex Nas. Only 5 plots have a single species. *Vaccinium vitis-idaea* Linn., followed by *Rhododendron dauricum* L. and *Rosa Bella* Rehd; *Vaccinium vitis-idaea* Linn., followed by *Rhododendron dauricum* L. and *Rosa Bella* Rehd; *Vaccinium vitis-ida* Linn., followed by *Rhododendron dauricum* L. and *Rosa Bella* Rehd; *Vaccinium vitis-ida* Linn., followed by *Rhododendron* L. and *Rosa Bella* Rehd; and 4. The most important values were *Vaccinium vitis-idaea* Linn., followed by *Rhododendron dauricum* L. and *Rosa Bella* Rehd; *Vaccinium vitis-idaea* Linn. for plot 5, followed by *Spiraea salicifolia* L. and *Rhododendron dauricum* L. for plots 6, 7 and 8, *Vaccinium vitis-idaea* Linn. was the most important value, followed by *Rhododendron dauricum* L. and *Rosa Bella* Rehd. The majority of common species indicates that the dominant species are comparatively similar^[17].

Herbaceous layer: The most important value of sample 1 was *Deyeuxia langsdorffii* (L. ink) Kunth., followed by *Rubia cordifolia* L. and *Maaianthemum bifolium* (L.) F. W. Schmidt; the most important value of sample 2 was *Rubia cordifolia* L., followed by *Fragaria orientalis* insk and *Deyeuxia langsdorffii* (L. ink) Kunth ii.; and the most important value of sample 3 was *Deyeuxia langsdorffii* (L. ink) Kunth ii. The second is *Rubia cordifolia* L. and *Trientalis europaea*; the maximum value of the four plots is *Deyeuxia langsdorffii* (L.

Table 2. The important valves of common species and single species in the shrub layer

No.	Species	Important valves(%)								Common species/Sin- gle species
		I	II	III	IV	V	VI	VII	VIII	
1	<i>Vaccinium vitis-idaea</i> Linn.	42.70	29.79	29.82	35.97	20.13	50.22	43.62	37.18	Common species
2	<i>Rhododendron dauricum</i> L.	22.10	29.14	28.94	25.07	14.18	12.57	18.96	35.90	
3	<i>Rosa bella</i> Rehd	12.80	8.83	25.14	5.09	9.49	4.43	11.80	5.00	
4	<i>Spiraea salicifolia</i> L.	14.00	6.91	4.67	0.83	14.81		3.80		
5	<i>Ribes nigrum</i> L.	1.70	2.08		2.05	10.01			2.80	
6	<i>Sorbaria sorbifolia</i> (L.) A. Br		4.56			4.26		0.90		
7	<i>Ledum palustre</i> L.				11.20		11.30		4.00	
8	<i>Rubus corchorifolius</i> L.f.	5.90		7.70						Single species
9	<i>Salix raddeana</i> Laksch. ex Nas.					2.40				

ink) kunth.; the second is *Maianthemum bifolium* (L.) F. W. Schmidt and *Rubia cordifolia* L.; the fifth plot is *Fragaria cordifolia* L. Losinsk, the second is *Deyeuxia langsdorffii* (L.) kunthia L.; the sixth plot is the most important value. *Fragaria orientalis* Losinsk was the largest, followed by *Deyeuxia langsdorffii* II (L ink) kunth. and *Rubia cordifolia* L.; *Linnaea borealis* L., followed by *Maianthemum bifolium* (L.), F. W. Schmidt and *Rubia cordifolia* L., and *Deyeuxia langsdorffii* II (L ink) kunth. The largest, followed by *Vicnasepium* and *Linnaea borealis* L., as a control. The maximum value of area 8 was *Linnaea borealis* L., followed by *Maianthemum bifolium* (L.) F. W. Schmidt and *Rubia cordifolia* L.

2.2 Diversity of Shrubs and Herbs under Different Transformation Methods

Simpson index reflects the concentration of the whole community. Shrub layer: Simpson index of sample 6 in 2012 is basically the same as that in 2017, and has little change. Simpson index of sample 5 in 2017 is lower than that in 2012, which differs greatly from other transformation measures. Simpson index of other sample sites in 2017 is higher than that in 2012; Shannon-Wiener index of sample 6 has little change compared with that in 2012, and Shannon-Wiener index of sample 5 has little change in 2012. The on-Wiener index is lower than that in 2017, which is quite different from other reformation measures.

The overall change trend of Shannon-Wiener index and Simpson index is similar after five years reformation; The richness index of No. 2 plot and No. 4 plot remained basically unchanged. The richness index of No. 5 and No. 6 plot was lower than that of the revamped plot in 2012. Unlike other plots, the richness index of the other plots in 2017 was higher than that of 2012; the evenness index of No. 2 plot and No. 4 plot basically changed little, the values of No. 5 and No. 6 plots were lower than that of the revamped plot in 2012, and the evenness index of other plots in 2017 After 5 years improvement, the overall change trend of richness index and evenness index is similar.

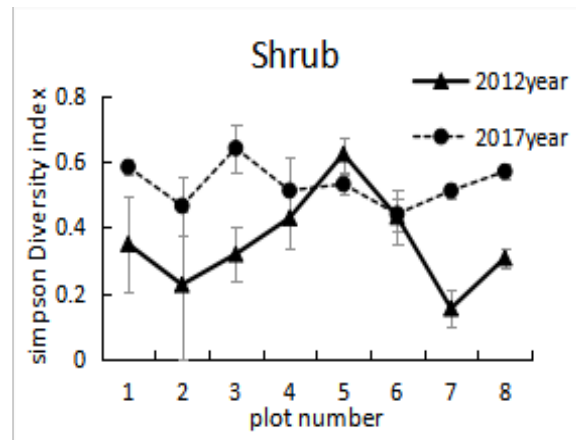
**Figure 1.** Two-year shrub Simpson

Table 3. Common species of herbaceous layer and a single important value

No.	Species	Important valves(%)								Common species/Single species
		I	II	III	IV	V	VI	VII	VIII	
1	Linnaea borealis L.	1.21	0.23	6.68	4.80	3.69	19.81	2.35	35.24	Common species
2	Deyeuxia langsdoerffii (Link) Kunth.	36.28	6.63	38.77	18.02	15.41	3.76	11.70	8.83	
3	Fragaria orientalis Losinsk	4.43	8.77	7.90	0.41	24.74	1.74	1.19	0.38	
4	Trientalis europaea	3.81	3.44	9.27	1.67	4.16	0.55	0.16	0.55	
5	Rubia cordifolia L.	26.55	8.78	12.94	5.39	14.70	6.84	0.27	6.84	
6	Maianthemum bifolium (L.) F. W. Schmidt	10.99	4.44	2.65	5.98	3.83	18.88	7.92	11.52	
7	Vicia sepium	5.13	0.67	0.75	0.56	0.13	1.28	3.46	1.27	Single species
8	Adina rubella Hance	0.76								
9	Convallaria majalis Linn.		2.28							
10	Paris verticillata M. Bieb.					0.90				
11	Adenophora tricuspidata (Fisch. ex Roem. et Schult.) A. DC.					3.50				
12	Potentilla chinensis Ser.							0.60		
13	Anemone dichotoma L.								0.29	
14	Anemone cathayensis Kitag								0.29	
15	Artemisia tanacetifolia Linn.								0.25	

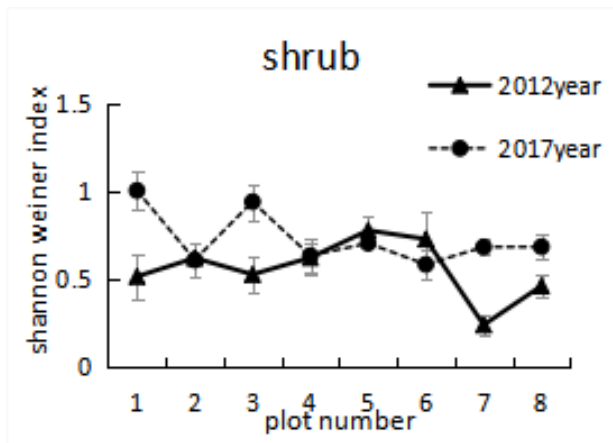


Figure 2. Two-year shrub Shannon-Wiener index comparison

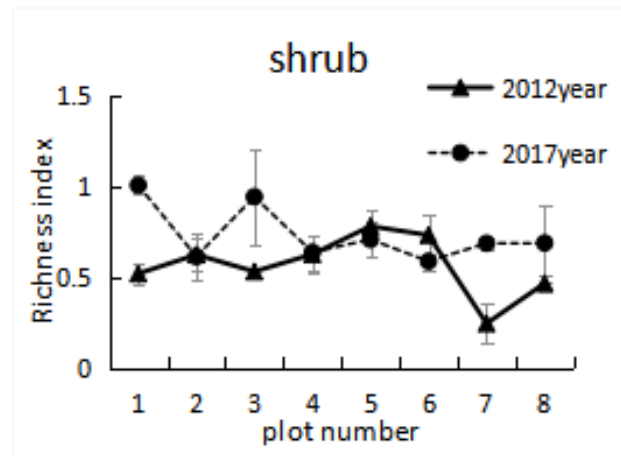


Figure 3. Two-year shrub richness

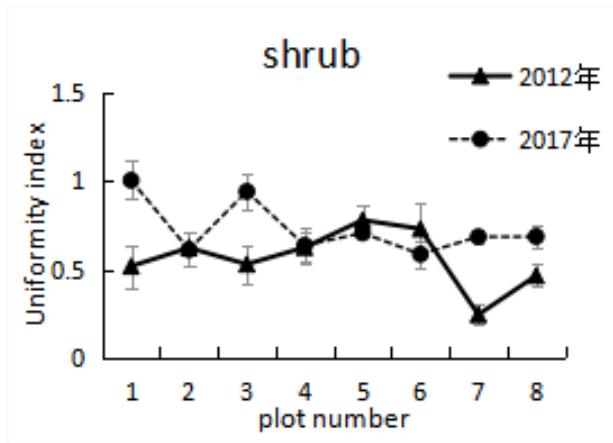


Figure 4.Two-year shrub uniformity index comparison

Herbaceous layer: Simpson index of sample 3 remained unchanged, Simpson index of sample 1 and 8 was lower than that of 2012 in 2017, Simpson index of other sample plots increased greatly in 2017; Shannon-Wiener index of sample 3 remained unchanged, Shannon-Wiener index of sample 1 and 8 were lower than that of 2012, Shannon-Wiener index of other sample plots increased in 2017; richness index of sample 4 was lower than that of 2012. Low, the richness index of No. 1, 2 and 5 plots has increased significantly, the richness index of No. 3, 6, 7 and 8 plots has a smaller growth than that of 2012, the evenness index of No. 4 plots has decreased compared with 2012, the evenness index of No. 1, 2 and 5 plots has greatly increased, the evenness index of No. 3, 6, 7 and 8 plots has a smaller growth than that of 2012, and the increasing trend of richness index is similar to that of evenness index.

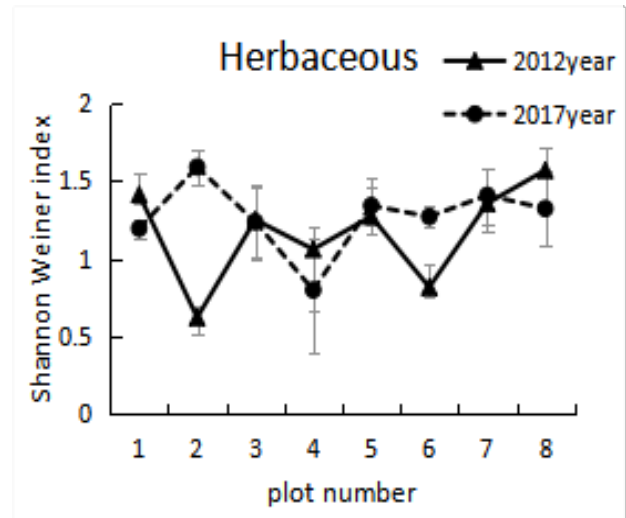


Figure 5.Two-year herbal Shannon-Wiener index comparison

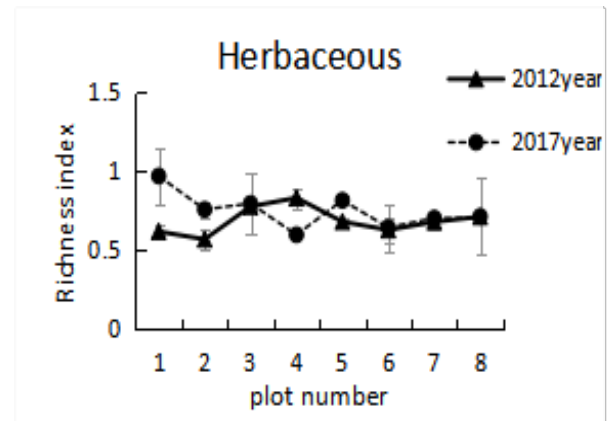


Figure 6.Two-year herbal richness



Figure 4.Two-year herbal Simpson

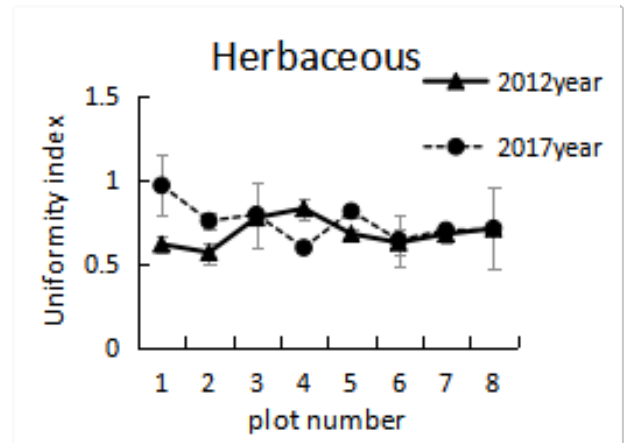


Figure 7.Two-year herb uniformity index comparison

Note: * At the significant level of 0.05, one-way ANOVA was performed.

2.3 Comparison of Biomass Values under Different Transformation Methods

From the following table (Table 2.), it can be concluded that the biomass of artificial regeneration in sample plot I decreased except litter layer, and the biomass of each layer in sample plot II increased significantly. The biomass of sample plot III decreased except litter layer, the biomass of each layer in sample plot IV decreased, the biomass of each layer in sample plot V decreased, and the biomass of each layer in sample plot VI increased. From the above, we can conclude that the biomass value of thinning intensity in the range of 30% to 40% is increasing annually, and the simple way of removing the ground cover can not effectively improve the biomass of undergrowth plants, and five years later, the value of litter layer is reversed. And bigger.

Table4 . Comparison of 2 years biomass under different transformation methods

Sample number	years	t·hm ⁻² Litter biomass	t·hm ⁻² Shrub layer biomass	t·hm ⁻² Herb layer biomass	t·hm ⁻² total
I	2012	2.8133	4.6211	0.4943	7.9287
	2017	6.4407	1.0041	0.3516	7.7963
II	2012	2.9659	1.3557	0.5678	4.8894
	2017	3.4119	3.5538	0.7082	7.6739
III	2012	3.3584	2.7813	1.1786	7.3183
	2017	3.3868	1.5188	0.5213	5.4268
IV	2012	4.1292	2.3636	1.0303	7.5231
	2017	3.3868	1.5188	0.5213	5.4268
V	2012	2.7567	1.8309	0.5998	5.1874
	2017	6.4094	2.0697	0.4956	8.9748
VI	2012	2.6643	2.2318	0.6030	5.4991
	2017	6.8243	2.3374	1.0035	10.1652
VII	2012	2.6856	2.4597	0.6113	5.7566
	2017	6.8243	2.3374	1.0035	10.1652
VIII	2012	2.6008	2.3407	0.9136	5.8551
	2017	8.5552	7.0843	0.5718	16.2113

3. Discussion

The effects of different management methods on plant species diversity, growth, development and regeneration of *L. gmelinii* forest are shrub layer: after different transformation methods, plant richness, growth uniformity and regeneration degree have been significantly improved. The biomass of shrub layer increased over 12 years, while that of herbaceous layer decreased. Because the tall *Rhododendron* in the upper layer blocked the sunshine, the growth of herbaceous under the forest was slow and the biomass was reduced. The transformation effect is in the order of I > II > V > III > VI > IV > VII.

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Key Technologies for Restoration and Function Improvement of Forest Ecosystem in Fire and Cutting-off Land

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EDUCATIONAL

Botanical Education: Management of Greening and Maintenance of Modern Landscape

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ABSTRACT

In the management of greening and maintenance of modern garden landscape, the prevention of natural disasters should be emphasized. Adequate fertilizer and water are provided to the plants to meet their growing needs. Pests and diseases are effectively prevented and controlled.

1. Introduction: Necessity and Characteristics of Management for Greening and Maintenance of Modern Garden Landscape

1.1 Effects on Plants

Modern garden landscape always consists of several different species of plants. The greening and maintenance for modern garden landscape means to maintain the healthy growth of those plants. Maintenance work in specific should be targeted and different maintenance methods should be selected according to the species of plant. Plants have their own life characteristics, and their maintenance methods are complex^[1]. The greening and maintenance of modern garden, in a sense, is equivalent to providing a better environment for plants to ensure a balanced growth. Therefore, spe-

cific maintenance conditions, such as humidity, soil and temperature, are needed to ensure the healthy growth of plants.

1.2 Effects on Overall Design

Greening and maintenance of a modern garden is a kind of plastic art, and the maturity of techniques can bring great changes in the overall aesthetics. In modern gardens, plants are rich in variety and diverse in color, and the overall shape is formed by a combination of many different factors. The degree of greening and maintenance also determines the form style of the garden landscape. It is believed that greening and maintenance of modern garden is a step in the shape design of gardens, and a work item generated to highlight the artistic conception different from other art forms.

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2. Problems of Management for Greening and Maintenance of Modern Garden Landscape

2.1 Systematic Issues on Management for Greening and Maintenance of Modern Garden Landscape

In China, this work has been doing well in some economically developed areas. However, in some other economically underdeveloped areas, due to the serious shortage of funds for garden maintenance, this work is done perfunctorily, as the destruction and occupation of vegetation occur constantly. These areas are ubiquitous of unsound management systems in greening and maintenance of modern garden landscape, with a poor performance in execution and lack of a complete set of assessment methods, thus leaving many projects half done.

2.2 Epistemic Issues on Management for Greening and Maintenance of Modern Garden Landscape

Only by fully completing greening and maintenance management of modern garden landscape in later stage can an originally expected result of greening for modern garden landscape being achieved, which will bring people a pleasing landscape and enjoyment for beauty. Meanwhile, its economic, ecological, social, scientific, educational and other functions can be maximized. Excellent maintenance management can consolidate the existing achievements of greening for modern garden landscape, stimulate people's enthusiasm to join and support the career of greening for modern garden landscape, and greatly improve the public's awareness of cherishing and protecting vegetation, thus promoting the constructive development of greening for modern garden landscape^[2]. If the garden maintenance or management is lacking or insufficient, the intent of modern garden construction will not be implemented, whose social value, ecological value and humanity value will not achieve a maximum efficiency. If modern garden designers' design intent cannot be fully reflected, the original spirit of construction is bound to lose.

2.3 Quality Issues of Maintenance Workers and Managers

At present, methods of greening and maintenance management of modern garden landscape are generally simple, that is, basic watering, simple pruning, daily sanitation cleaning, etc., with low technical content. Due to lack of necessary technical training for maintenance managers, and general maintenance management company has very few professional staffs, most of the recruited workers are

employed after simple training. Their technical and professional quality is generally low, who could only do the maintenance work step-by-step without any innovation, and lead to a poor effect and low efficiency of maintenance. They are usually only responsible for maintaining plants' life, and make the least effort to ensure the plants are alive.

3. Measures for Greening and Maintenance Management of Modern Garden Landscape

3.1 Improve the Management System for Greening and Maintenance of Modern Garden Landscape

Maintenance and management works need better management standards and technical specifications. To grasp all aspects of maintenance work, it is necessary to formulate complete and detailed maintenance management measures. Greening areas with poor maintenance level and inadequate management should also be analyzed in time to find out the reasons. Greening areas with excellent maintenance measures and outstanding management should be summarized, promoted and demonstrated. Inviting professional teachers to train relevant personnel at regular intervals and encouraging innovation and practice to improve their professional skills of maintenance. Meanwhile, training in modern garden technology should also be carried out so that employees could master the rules of maintenance management and plant-protection techniques such as watering, fertilization, pruning, as well as prevention and control of pests, thereby practical problems in greening and maintenance can be well resolved^[3].

3.2 Enhance the Public's Protection Awareness of Vegetation

The construction of modern gardens is not easy, whose consolidation and maintenance are even harder. The greening and maintenance management of modern garden landscape needs the close cooperation of the masses. Various ways such as media, newspapers, radio, internet and billboards should be utilized to promote the glory of cherishing and protecting vegetation, and lower the shameful behavior of destroying it, thus raising the awareness of the masses to care for the ecological environment. At the same time, it is necessary to strengthen the legal education propaganda and strive to raise the legal awareness of the whole people. Through various forms such as voluntary tree planting, adoption of Greening areas or trees and flowers, it evokes the concern and care of the whole people for ecological construction, motivates people to partic-

ipate in it and cultivates citizens' enthusiasm to care about and love the Greening environment, which make people voluntarily devote themselves into actions of greening and beautifying the environment, and create an atmosphere in which a beautiful homeland is preserved.

3.3 Maintain Gardens in Accordance with the Maintenance Management Guidelines

(1) Trees should be regularly brushed with lime and sprayed with insecticide for disinfection and pest control, and be properly trimmed as needed. (2) Care work should be carried out in strict accordance with the order of watering, fertilization, weed removal, loosening, pruning, de-worming, and disease healing. (3) For the maintenance of plants, we advocate the artificial control and biochemical control, and the dosage of pesticides in use should be subjected to inducing no harm to human health and the environment. The use of pesticides should be done in one time in clear and windless weather, in case the spread of pesticides causes harm to humans and the environment. (4) Fertilization can be moderately implemented according to the growth of the plant, and the appropriate fertilization should be implemented based on the actual needs of plants.

3.4 Choose Native Species for Priority

In the choice of plants, native plants should be preferred for planting. Appropriate native plants are selected according to the climatic characteristics and soil conditions of different places. Since native plants can better adapt to the local soil and environment, they can survive better than those introduced in the field. In addition, the cultivation of native plants can also better represent the local characteristics and distinguish them from other garden landscapes. Meanwhile, the selection of a large number of local ground cover plants can effectively save costs and increase profits. The different niches of native plants can meet the various requirements of garden landscapes, and can also effectively reduce the invasion of alien species, which is conducive to the coordinated development of natural development^[4].

3.5 Put Eco-configuration into Practice

The mastery of environmental factors is a prerequisite for garden landscape planting. Putting Ecological configuration into practice means selecting suitable ground cover plants according to the actual conditions of environment to fulfill their growth efficiency, so that the overall plants condition can be best optimized. Environmental factors such as terrain and ecological conditions should be inves-

tigated on the spot to ensure the survival rate of plants in this environment.

3.6 Collocate Various Plants Reasonably

The combination of plants is also one of the important factors affecting the maintenance of landscape gardens. Ground cover plants are generally distributed at the bottom of the plant community, and play a role in setting off the best combination of plants such as shrubs. In the case where the branch point of the upper plant is low, the ground cover plant sticking to ground can be selected, and when it is higher, the ground cover plant with higher plant height can be selected. In capacious planting areas, shrubs with a sparse upper layer can be collocated with higher ground cover plants, while in narrow planting areas, shorter ground cover plants can be planted. Work in this respect will also greatly improve the maintenance level.

4. Methods of Management for Greening and Maintenance of Modern Garden Landscape

4.1 Fertilization

The arbor tree is tall, with a developed root system that spreads deeply and widely in the ground. When planting, it is necessary to open a large hole with a square of 0.8-1.0 cubic meters, and fill it with fertile soil, 30 cm above the flat land. In terms of fertilization, the fertilizer type is mainly compound fertilizer, and the ratio of N, P, and K for the young trees of 1-3 years old is 5:3:2, for the trees over three years is 3:2:1. Within three years after planting, fertilization should be applied once every spring, summer and autumn. Each time, 1-2kg of compound fertilizer will be used, and small trees will be applied less while big trees, more. The method of fertilization is as follows: the small tree is applied with liquid fertilizer, combined with losing its soil, and the big tree is uniformly dry applied to the ground in the crown width. The tall trees of three years or more may not be fertilized in principle. The shrub tree is small, mainly planted in dentpits or shallow ridges, with shallow clumps of roots. The appropriate amount of compound fertilizer is applied according to the soil and tree vigor. Liquid application is combined with dry application. For flower and fruit shrubs, the P and K fertilizer should be properly increased, and for foliage shrubs, the N fertilizer be increased.

4.2 Irrigation

In addition to factors such as planting methods, habits of tree species and climate, irrigation should also take soil types into consideration. For saline-alkali soil, irrigation

should be combined with intervillage loose soil as much as possible, preferably using river water. Considering that the sand has poor water retention and leaks easily, the frequency of irrigation for sand land should be increased appropriately. For sand land, the frequency of irrigation should be increased appropriately with a small amount of water at a time. Organic fertilizer should be applied to increase water retention and fertility. Bottomland should also be poured with small amount of water without any accumulation, and alkali prevention and drainage are concerned. Heavy clay soil has strong water retention, for whom the frequency and amount of irrigation should be both reduced and organic fertilizer and river sand should be applied to increase its permeability.

The water management of trees should focus on young trees, based on the principle of moisturizing without stains and the topsoil is dry but not white. Tall trees are thick with leaves and deep-rooted, whose growth will not be affected by water shortage. Shrubs are short with shallow root systems. Whether potted or planted in the field, they must be protected from drought and kept in moisture without stains, so that they can grow normally.

4.3 Prevention and Control of Diseases and Pests

Tree beetles, spiders, scarabs, etc., are the main pests of trees. Although conventional pesticides can prevent and treat them, chemical pesticides should be used in a reasonable way. Meanwhile, biological and physical methods should be actively carried out, and various control methods should be used in combination. Considering a variety of factors such as control effects, economic benefits, social security and ecological balance, pests should be controlled within the allowable range, rather than killing them all.

4.4 Pruning Trees

Trees use photosynthesis to gain energy through leaves. Trimming trees too much will weaken its overall vitality and is not conducive to the growth of trees. Excessive deep pruning provides a path for fungal and bacterial infections while causing serious damage to trees. Therefore, the trimming of trees should be reduced.

4.5 Maintain Garden Trees for Greening

Through art design and careful management, trees for greening could grow in balance with bunch of large branches. This is one of the keys to the success of greening that will allow plants remain high inside and low outside, and form the natural and plump spherosomes scattered delicately. Arbor trees are required to have tall and straight trunks. Therefore, the old branches should be

removed in a planned schedule to cultivate new branches. Adult trees should be cut off irregular branches in time, which will be broken in case of heavy wind and rain, and make roots to be uprooted and cause losses in more severe cases. While those little branches with large crown and thick leaves should be kept to resist the wind. Shrubs are required to be neat and orderly with shape, which is formed through continuous growth and artificial trimming. The shape of shrubs can be trimmed into spheres, squares, fans, mushrooms abstract patterns, etc.. You can even use iron wire to weave text, "two dragons frolicking with a pearl" and "peacock spreads its tail" to allow branches and leaves of trees grow in them and trim to form the shape. Whether arbor trees or shrubs, it is necessary to clean up the spent flowers and fruits in time to avoid the consumption of nutrients.

4.6 Maintain Garden Grasslands for Greening

The principle of grasslands maintenance is: homogeneous and uniform, pure without stains and evergreen in all seasons. In general, grasslands for greening can be divided into four stages according to the length of planting time. The first stage ranges from just-planted to fully-grown, which refers to a period (approximately one year) in which the freshly planted grass grow to full coverage (100% coverage without vacancy), and is also named as fully-grown period. The second stage is the prosperous growth stage, which refers to 2-5 years after planting, and is also named as prosperous growth period. The third is the slow growth stage, which refers to 6-10 years after planting, and is also named as slow growth period. The fourth is the degradation stage, which refers to 10-15 years after planting, and is also named as degradation period. The growth rate of grassland decreased after 6-10 years of planting, and the key emphasis in work turns to pay attention to the prevention and control of pests. Push aside the grass during inspection to detect larvae and it is necessary to find them out in time and apply pesticide at their young age. Remove the hay that has been harmed three days later, and replenish the spot with urea solution, then the growth resumes after a week

4.7 Maintain and Manage Hedgerows

The hedgerow has higher requirements for fertilizer and water conditions. When the hedgerow is first planted, it is necessary to mix the appropriate amount of decomposed organic fertilizer in the soil, so that it grows fast after planting. The principle of fertilization is: sufficient base fertilizer and speedy replenishment, and fertilizer must be applied after cutting. Root fertilization is also carried out

when necessary. Irrigation management is mainly focused on moisturizing to make sure that the topsoil is dry but not white. Drain away water after rain to avoid accumulation so as not to cause rotten roots and affect growth.

4.8 Maintain and Manage Potted Flowers

In garden greening industry, potted flowers mainly refer to two types: potted seasonal flowers and potted shade plants. Potted flowers are cultivated in two stages. The first stage is to cultivate in the flower nursery, and the second stage is to cultivate in the pots and let it grow till its ornamental value arises, or it is close to blossom, then put it in the green spots to embellish the life. In addition, there are a small number of finished pot culture in garden greening industry, which is placed in the scenic spots of special need. The cultivation management is roughly the same with the wooden potted flowers. The key point of difference is the trimming and shape preserving. Different pot cultures have different artistic shapes. Without trimming, the original design style will be lost. The trimming for pot culture needs to be operated by technicians or gardeners with professional knowledge and skills, and should not destroy the viewing surface and lose the original genre, style and artistic shape.

5. Conclusion

In summary, garden workers should consider plants' demands for water in different conditions such as climates and soil, carefully observe changes in climatic environment, fully master the characteristics of various types of trees, and take reasonable measures in a targeted manner. Water should be supplied regularly and quantitatively to ensure the normal growth of the trees in various periods to fully display their seasonal beauty and spread the urban civilization.

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